Historic, Archive Document

Do not assume content reflects current scientific knowledge, policies, or practices.



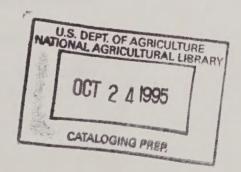
United States
Department of
Agriculture

Diffuse and Spotted Knapweed Biological Control Project Manual

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

Biological Control Operations



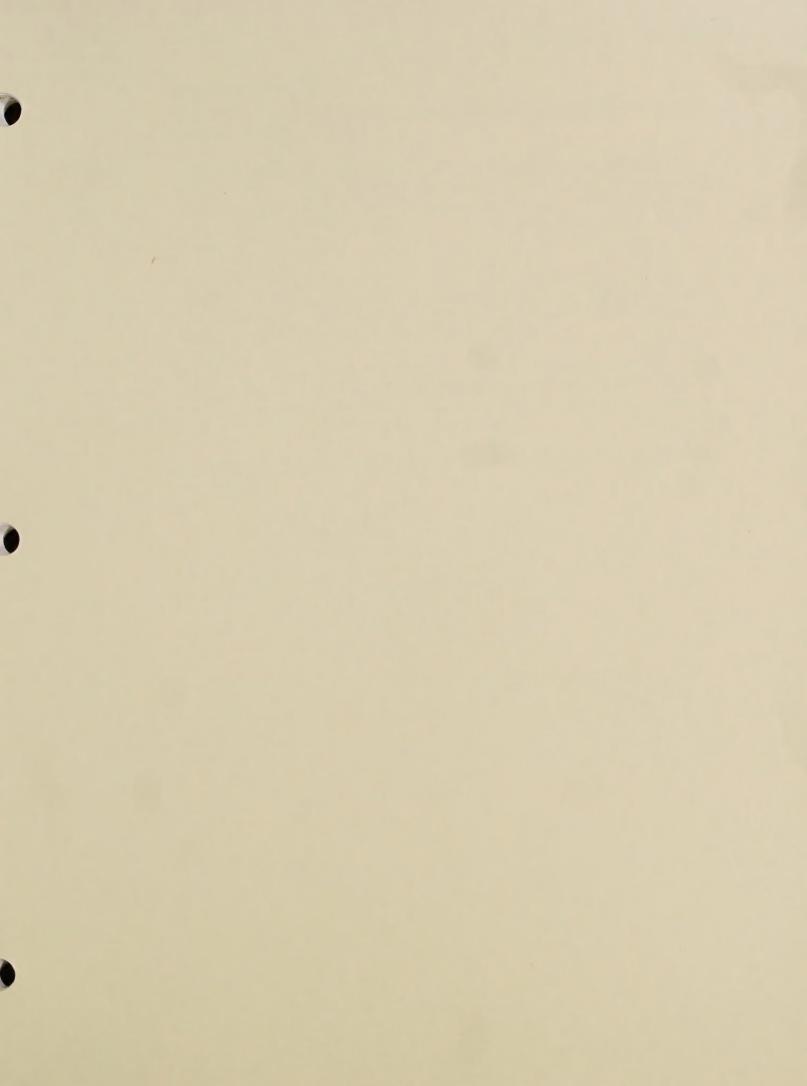
CONTENTS

	Page
INTRODUCTION	
Orientation to the Biological Control Project Against Diffuse and Spotted Knapweed (DSK Project)	1.1
Roles and Responsibilities	1.5
How to Use This Manual	1.7
ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)	
Introduction	2.1
Select a Suitable Site for the Insectary	2.3
Information Sheet (FISPIS)	2.5
Release the Biocontrol Agents at a Phase 1 FIS	2.7
Fill Out Forms AD-943 (Biological Shipment	
Record-Non-Quarantine) and AD-943A (Supplemental Data)	2.15
Collect Samples of Biocontrol Agents From the	
Phase 1 FIS	2.17
Fill Out a Diffuse and Spotted Knapweed Biocontrol Agent Recovery and Sampling Report	2.27
Agent Recovery and Sampling Report	2.21
ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS)	
Introduction	3.1
Select a Suitable Site for the Insectary	3.3
Collect Biocontrol Agents From a Phase 1 FIS for Redistribution to a Phase 2 FIS	3.5
Release the Biocontrol Agents at a Phase 2 FIS	3.19
APPENDIXES	
Appendix 1: Diffuse and Spotted Knapweed Plants	4.1
Appendix 2: Biology and Identification of Biocontrol Agents	5.1
Appendix 3: Summary of Diffuse and Spotted Knapweed	3.1
Biocontrol Agents	6.1
Annandiy A: Operation of Transpak II Global	
Positioning System (GPS) Units	7.1
Appendix 5: APHIS Process to Release Exotic Natural	8.1
Enemies of Weeds for Establishment and Redistribution	9.1
Appendix 7: Host Plant Specificity Testing	10.1
Appendix 8: Collection Vacuum	11.1
	10.1
INDEX	12.1
COMMENT SHEET	13.1

PHOTOGRAPHS

	Number
Spotted and Diffuse Knapweed Plants	
Centaurea maculosa Lam. (spotted knapweed) flower headed knapweed plant, showing branching form	1-2
Spotted knapweed infested range	1-3
Spotted knapweed rosette	1-4
Mature spotted knapweed seedheads	1-5
Centaurea diffusa Lam. (diffuse knapweed) uncommon purple flower head	. 1-6
Diffuse knapweed normal white flower head	1-7
Diffuse knapweed plant showing branching and form	1-8
Diffuse knapweed infested range	1-9
Biocontrol Agents	
Agapeta zoegana adult root boring moth	2-1
Agapeta zoegana larva and root feeding damage	2-2
Pterolonche inspersa adult root boring moth	2-3
Cyphocleonus achates adult root boring weevil	. 2-4
Cyphocleonus achates larvae and root feeding damage	. 2-5
Larinus minutus adult seedhead weevil	2-6
Larinus minutus adult emergence hole in knapweed seedhead	. 2-7
Larinus obtusus adult seedhead weevil	2-8
Bangasternus fausti adult seedhead weevil	. 2-9
Metzneria paucipunctella adult seedhead moth	. 2-10
Metzneria paucipunctella larva and seedhead feeding damage	. 2-11
Urophora affinis adult seedhead fly on plant gall	. 2-12

	Number
Urophora affinis larva and gall in knapweed seedhead	. 2-13
Urophora quadrifasciata adult seedhead fly	. 2-14
Urophora quadrifasciata gall compared to knapweed seed	. 2-15
Chaetorellia acrolophi adult seedhead fly	. 2-16
Terellia virens adult seedhead fly	. 2-17
Sphenoptera jugoslavica adult root boring beetle on diffuse knapweed	. 2-18
Sphenoptera jugoslavica larva and root feeding damage	. 2-19





INTRODUCTION

Orientation to the Biological Control Project Against Diffuse and Spotted Knapweed (DSK Project)

History of Diffuse Knapweed

Diffuse knapweed (*Centaurea diffusa*), a native of Eurasia, was introduced into British Columbia, Canada, in the late 1800's. It was discovered in the Pacific Northwest of the United States by the early 1900's. Diffuse knapweed is found on waste land and maritime sand from southern Europe to the north central Ukraine and into central Europe.

Diffuse knapweed disperses by seed, the plants breaking off and tumbling in the wind. The seed is also spread by vehicles, crop seed, hay and people. Although intensive cultivation controls diffuse knapweed in crop lands, it continues to invade range and wildlife foraging areas that are not conducive to cultivation.

Chemical control is effective, but the costs are prohibitive, and some knapweed grows in environmentally sensitive areas. The integration of chemical control and range management practices with biological control strategies may prove to be a necessary and satisfactory method of controlling diffuse knapweed.

The following insects are currently being used as biological control agents of diffuse knapweed:

- Bangasternus fausti (a seedhead weevil)
- Cyphocleonus achates (a root boring weevil)
- Larinus minutus (a seedhead weevil)
- Pelochrista medullana (a root boring moth)
- Pterolonche inspersa (a root boring moth)
- Sphenoptera jugoslavica (a root boring beetle)
- Urophora affinis (a seedhead fly)
- Urophora quadrifasciata (a seedhead fly)

Using a combination of these insects will probably be the best overall strategy for diffuse knapweed biocontrol. *Sphenoptera jugoslavica* has shown control at sites in Idaho, Washington, and South Dakota. Although no new biocontrol agents currently are being studied for importation into North America, some agents are still being imported from Europe to start insectaries. Any insects that are imported are tested for pathogens and parasites to assure healthy stock.

History of Spotted Knapweed

A native of Eastern Europe and Asia, spotted knapweed (*Centaurea maculosa*) was introduced into North America in the early 1900's. The weed spreads rapidly; it was found in one county in Montana in 1920, and is now found in all 56 counties of that State.

Spotted knapweed is controlled in crop land areas by cultivation. Livestock and wildlife will feed on the rosettes, but as the plant matures it becomes less palatable, and the animals cease feeding on the plants.

Chemical control of spotted knapweed with various herbicides is one of the more widely used options. However, the use of herbicides over rough terrain and near waterways is limited, and the cost of herbicide applications to large areas becomes prohibitive. As with diffuse knapweed, integration of chemical control and range management practices with biological control strategies may prove to be the best method of control.

The following insects are currently being used (or have been used) as biological control agents of spotted knapweed:

- Agapeta zoegana (a root boring moth)
- Bangasternus fausti (a root boring weevil)
- Chaetorellia acrolophi (a seedhead fly)
- Cyphocleonus achates (a root boring weevil)
- Larinus minutus (a seedhead weevil)
- Larinus obtusus (a seedhead weevil)
- Metzneria paucipunctella (a seedhead moth)
- Pelochrista medullana (a root boring moth)
- Pterolonche inspersa (a root boring moth)
- Sphenoptera jugoslavica (a root boring beetle)
- Terellia virens (a seedhead fly)
- Urophora affinis (a seedhead fly)
- Urophora quadrifasciata (a seedhead fly)

By itself, it is doubtful that any one these insects could successfully control spotted knapweed. However, a combination of biological control agents may achieve significant results. Although no new insects are being screened for release in the United States for control of spotted knapweed, some approved biocontrol agents are still being imported from Europe to establish insectaries in the United States. Before these insects are imported, they are carefully tested for pathogens and parasites. We want to assure we have healthy populations of biocontrol agents.

Economics

Diffuse and spotted knapweed have caused serious problems for ranchers in Western and Midwestern States. These weeds are aggressive, short-lived perennials that displace other plants in pasture and rangeland habitats. Reductions of forage from 10 to 100 percent have been observed. Most range animals and wildlife avoid diffuse and spotted knapweed, especially when the weeds are in the mature stage.

Project Length

Project activity is scheduled for fiscal years (FY) 1995 and 1996.

FY 1995: In selected States in PPQ's Northeastern, Central, and Western Regions, the following will be accomplished:

- Selection of new insectary sites
- Evaluation of previous releases
- Release of new agents
- Redistribution of established biocontrol agents

FY 1996: The previous accomplishments for FY 1995 will be repeated in selected States in PPQ's Northeastern, Central and Western Regions, but with decreasing emphasis on selection of new insectary sites, and increasing emphasis on redistribution of established biocontrol agents.

Who's Involved

The Agricultural Research Service (ARS), State departments of agriculture, universities, Extension Service and industry personnel, and other Animal and Plant Health Inspection Service (APHIS) staffs are contributing to the implementation of the DSK Project.

The Project Leader is Paul Parker, who is located at the National Biological Control Laboratory, PPQ, APHIS, in Mission, Texas.

PPQ Entomologists are Robert Richard, Ronald Lang, and Richard Hansen, who are located at the Bozeman Biological Control Facility (BBCF), in Bozeman, Montana.

Cooperators include PPQ line personnel, State departments of agriculture personnel, university researchers, and Extension Service personnel. State project coordinators are generally State plant health directors (SPHD's) or officers-in-charge (OIC's) at designated PPQ locations.

Goal and **Objectives**

The goal is to provide effective, economical methods for control of diffuse and spotted knapweed using biological control. The project objectives cover the following topics:

- Distribution of diffuse and spotted knapweed
- Establishment of field insectary sites
- Collection of biological control agents
- Release of biological control agents
- Recovery of biological control agents Importation of biological control agents
- Redistribution of biological control agents
- Development of predictive models
- Education and public awareness of diffuse and spotted knapweed
- Laboratory mass rearing of biological control agents
- Evaluation of diffuse and spotted knapweed biological control
- Cooperator involvement and personnel training
- Economic impact of diffuse and spotted knapweed

INTRODUCTION Roles and Responsibilities

Cooperators

PPQ line personnel, State departments of agriculture personnel, university researchers, and Extension Service personnel will provide the technical assistance in the field to do the following tasks:

- 1. Select new insectary sites.
- 2. Evaluate previous releases.
- 3. Release new agents.
- 4. Redistribute established biocontrol agents.

State Project Coordinators

State project coordinators are generally State plant health directors (SPHD's) or officers-in-charge (OIC's) at designated PPQ locations. They are responsible for the following tasks:

- 1. Assign cooperators designated work areas.
- 2. Plan and coordinate work hours needed for cooperators to accomplish the tasks listed above.
- 3. Evaluate information gathered by cooperators to ensure accuracy and completeness.
 - 4. Communicate with the Project Leader about the project's progress.

Project Leader

Paul Parker

USDA, APHIS, PPQ

National Biological Control Laboratory

P. O. Box 2140

Mission, TX 78572

Commercial: (210) 580-7301

FAX: (210) 580-7300 E-Mail: !a348bcmissio

The Project Leader's responsibility is to coordinate all efforts while meeting the objectives of the project.

Bozeman Robert Richard
Biological Ronald Lang
Control Richard Hansen
Facility USDA, APHIS, PPQ
(BBCF) Forestry Sciences Lab

Montana State University Bozeman, MT 59717-0278 Commercial: (406) 994-5033

FAX: (406) 994-6591 E-Mail: !a348bcbozema

If you have any questions about the Biological Control Project Against Diffuse and Spotted Knapweed, make BBCF your first contact.

INTRODUCTION How to Use This Manual

Use the DSK Project Manual as an on-the-job reference when selecting new insectary sites, evaluating previous releases, releasing new agents, and redistributing established biocontrol agents.

Each tabbed section is independent, containing step-by-step procedures.

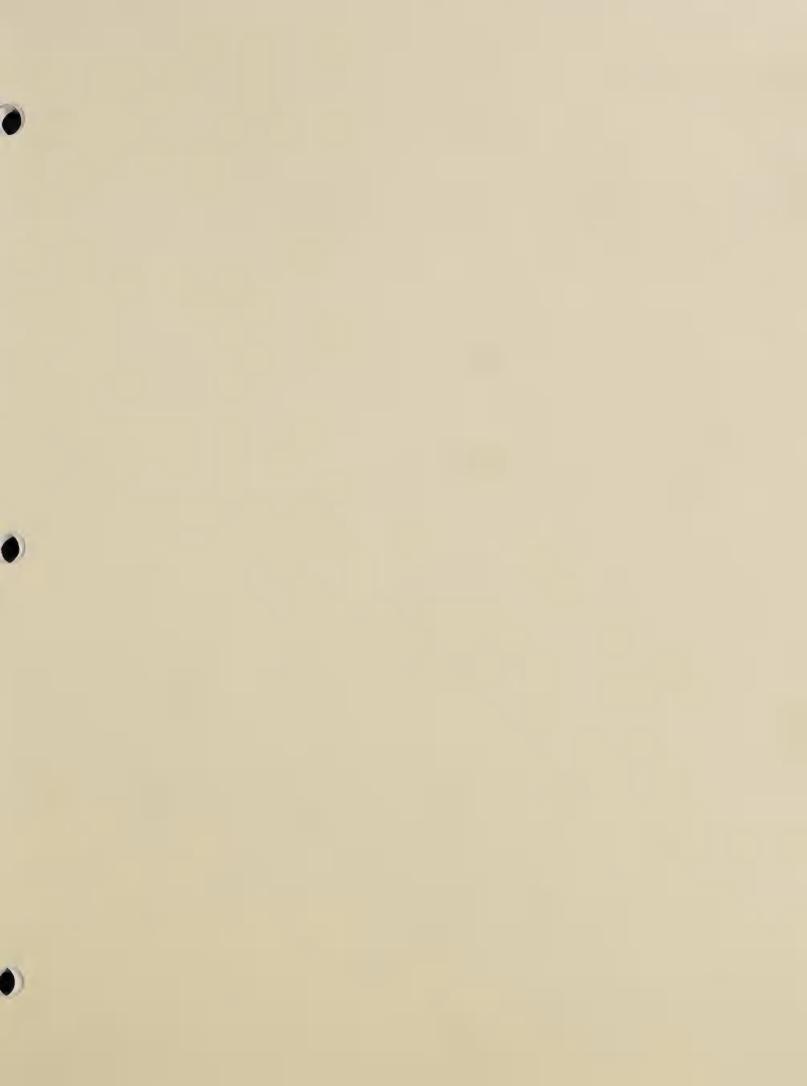
Each section has an Introduction which contains general information relating to the section's main content.

The Overview is a list of steps described in the section. If you are familiar with the steps, you can use the Overview as a checklist.

Use the Appendixes as they relate to the other sections of the manual. In some places an Appendix is referenced; in other places it is assumed that you accessed an Appendix to get the necessary information.

If the Contents is not specific enough, use the Index to find a topic and its page number.

Diffuse and Spotted Knapweed





ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS) Introduction

When to Establish

The Bozeman Biological Control Facility (BBCF) will contact you to let you know when the time is right for establishing a field insectary in your State.

Purpose

The purpose of a field insectary is to develop a field location where biological control agents can be collected in large numbers. The desired goal is to redistribute these agents to other infestations of diffuse and spotted knapweed, where the agents have not been released in the past or where high populations of the agents have not developed. For a description of the APHIS process to release exotic natural enemies of weeds for establishment and redistribution, see Appendix 5.

Overview

If you are familiar with the process, you can use the following overview as a checklist of the tasks for establishing a field insectary.

- 1. Select a suitable site for the insectary.
- 2. Fill out a Field Insectary Site Preliminary Information Sheet (FISPIS).
- 3. Release the biocontrol agents at the Phase 1 FIS.
- 4. Fill out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).
- 5. Collect samples of biocontrol agents from the Phase 1 FIS.
- 6. Fill out a Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS) Select a Suitable Site for the Insectary

Introduction

You must plan very carefully when you select a site for establishing a field insectary. You must be able to identify spotted and diffuse knapweed and determine if the site is suitable by considering all the criteria listed in Step 2 below. For help in identifying spotted and diffuse knapweed plants, see Appendix 1. BBCF will contact you to let you know when the time is right for establishing a field insectary in your State.

- Step 1 Contact State department of agriculture personnel and county extension educators to determine where large stands of spotted and diffuse knapweed are located.
- Step 2 Visit prospective sites to evaluate their suitability for establishing a field insectary, using the following criteria:
 - Stand density:
 - -- Moderately dense stands of spotted and diffuse knapweed are best. Some bare ground is desirable.
 - Sunlight:
 - -- Open fields without shade are best (the biocontrol agents like sun).
 - Exposure:
 - -- Southern exposures are best. Neutral exposures (flat locations) are acceptable, while northern exposures are least desirable.
 - Soil type:
 - -- Moderately textured loams are best. Clay soils are not as good.
 - -- The soil should be moderately to well drained, and the area should be free from seasonal flooding.
 - Grazing:
 - -- The area should be free from grazing.
 - Field size:
 - -- The field should be at least 2 acres large, preferably larger.
 - Pesticide use:
 - -- The site should not be exposed to insecticides.
 - -- No herbicides should be used within 500 ft. of the release point.
 - Land ownership:
 - -- Public land is usually a better choice than private land, because of longevity of management.
- Step 3 After you have visited several prospective sites, choose a site that has most of the desirable characteristics listed above.
- Step 4 Contact the land owner or land manager in person or by telephone. Identify yourself (give a business card if available).
- Step 5

 Ask permission to establish a field insectary site. Make sure that the land owner or land manager is aware of, and willing to make the 5-year commitment described in item 25 on the Field Insectary Site Preliminary Information Sheet.
- Step 6 Go to the next section--Fill Out a Field Insectary Site Preliminary Information Sheet.

BCO 05/95-01

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS) Fill Out a Field Insectary Site Preliminary Information Sheet (FISPIS)

Introduction

The FISPIS was designed to serve the following two basic functions (see an example in Appendix 6):

- 1. The FISPIS can aid in the "pre-season" process of selecting FIS release locations. For example, if 10 locations are being considered for 5 insect releases, the information contained in the FISPIS can help select the best locations for the releases.
- 2. The FISPIS provides a variety of useful biological and physical information about the release location that, when later coupled with FIS insect population data, can determine what site characteristics are best suited for biocontrol agents. This information can help guide the placement of future insect releases. If you have not already done so, complete this form at the time you release biocontrol agents at a Phase 1 FIS.
- Find the green form titled USDA-APHIS BIOCONTROL OF WEEDS: Field Insectary Site Preliminary Information Sheet (FISPIS). It should be included with the material you received from BBCF.
- **Step 2** Record the following information on the FISPIS:
 - Target Weed: Place an "X" in the Appropriate Space for Spotted or Diffuse Knapweed.
 - Release Code: Leave blank; this will be assigned by BBCF.
 - Contact Person: Record your name, address, and phone number.
 - Legal Land Owner: Record the land owner's name, address, and phone number.
 - Site Location: Select a site name, and identify the State, county, township, range, section, and quarter-section of your site. Determine and record the latitude and longitude of your site using the Transpak II Global Positioning System (GPS) unit (see Appendix 4). The manufacturer has provided detailed instructions for operating the unit. Please review these instructions before you take any readings.
- Step 3 Draw a map on the back of the FISPIS, or attach a map that shows road access to the site.
- Step 4 Record on the FISPIS the requested data pertaining to physical, biological, cultural, and other site characteristics. If you are not sure how to answer a question, leave it blank.

Step 5 Distribute the FISPIS as follows:

If you are:	Then:
A State plant health director (SPHD)	 RETAIN a photocopy of the FISPIS. MAIL the original to BBCF within 1 week of your release along with Forms AD-943 and AD-943A.
An officer-in-charge (OIC)	 RETAIN a photocopy of the FISPIS. MAIL a photocopy to your SPHD. MAIL the original to BBCF within 1 week of your release along with Forms AD-943 and AD-943A.
A PPQ officer	 RETAIN a photocopy of the FISPIS. GIVE a photocopy to your OIC. MAIL the original to BBCF within 1 week of your release along with Forms AD-943 and AD-943A.
A State cooperator	 RETAIN a photocopy of the FISPIS. MAIL a photocopy to the SPHD covering your State. MAIL the original to BBCF within 1 week of your release along with Forms AD-943 and AD-943A.

Step 6 Go to the next section--Release the Biocontrol Agents at a Phase 1 FIS.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS) Release the Biocontrol Agents at a Phase 1 FIS

Introduction

BBCF will work with you in choosing when and where to receive your shipment of biocontrol agents. If there is a suitable delivery point closer to the release site than your office, BBCF may opt to ship the biocontrol agents to that location. See Appendix 2 for photographs and narrative descriptions of the biocontrol agents. You can find information on the insects' life cycles and how they damage diffuse and spotted knapweed plants in Appendix 2. See Appendix 3 for a pronunciation guide.

Releasing Agapeta zoegana

<u>Step 1</u>:

Upon receipt from BBCF, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THIS BIOCONTROL AGENT THE SAME DAY YOU RECEIVE IT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pace in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

<u>Step 3</u>:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the moths are alive, sacrifice two or three for your voucher sample.

Step 6:

Pin the moths immediately upon return to your office.

Step 7:

Write the name of the biocontrol agent on a small paper label and place it on the pin with the insect. By doing this, you will have identified specimens to use as a reference when you collect moths in successive years for the recovery report.

Step 8:

Release the biocontrol agents on spotted knapweed within a 3-foot radius of the stake.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 10:

Go to the next section--Fill Out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

Releasing Bangasternus fausti and Larinus spp.

<u>Step 1</u>:

Upon receipt from BBCF, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°- 50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by securely driving a metal stake into the ground.

<u>Step 5</u>:

Count and retain dead biocontrol agents for voucher samples. If all of the beetles are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the beetles you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Write the name of the biocontrol agent on a small paper label and place it **inside** the vial. By doing this, you will have identified specimens to use as a reference when you collect beetles in successive years for the recovery report in. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on spotted or diffuse knapweed (except L. obtusus: release only on spotted knapweed) within a 3-foot radius of the stake.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 10:

Go to the next section--Fill Out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

Releasing Cyphocleonus achates

Step 1:

Upon receipt from BBCF, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the weevils are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the weevils you retained in a glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Write the name of the biocontrol agent on a small paper label and place it inside the vial. By doing this, you will have identified specimens to use as a reference when you collect weevils in successive years for the recovery report. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on spotted or diffuse knapweed plants within a 3-foot radius of the stake. Take time to hand place three to four weevils per plant.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 10:

Go to the next section--Fill Out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

Releasing Chaetorellia acrolophi, Terellia virens, and Urophora spp.

<u>Step 1</u>:

Upon receipt from BBCF, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather.

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the flies are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the flies you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Write the name of the biocontrol agent on a small paper label and place it **inside** the vial. By doing this, you will have identified specimens to use as a reference when you collect flies in successive years for the recovery report. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on diffuse and spotted knapweed plants within a 3-foot radius of the stake.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 10:

Go to the next section--Fill Out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

Releasing Metzneria paucipunctella

Step 1:

Upon receipt from BBCF, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the moths are alive, sacrifice two or three for your voucher sample.

Step 6:

Pin the moths as soon as you return to your office.

Step 7:

Write the name of the biocontrol agent on a small paper label and place it on the pin with the moth. By doing this, you will have identified specimens to use as a reference when you collect moths in successive years for the recovery report.

Step 8:

Release the biocontrol agents on spotted knapweed within a 3-foot radius of the stake.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 10:

Go to the next section--Fill Out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

Releasing Sphenoptera jugoslavica

Step 1:

Upon receipt from BBCF, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

<u>Step 3</u>:

Drive to the release site. DO NOT wait for good weather.

Step 4:

Mark the release point by driving a metal stake securely into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the beetles are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the beetles you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Write the name of the biocontrol agent on a small paper label and place it inside the vial. By doing this, you will have identified specimens to use as a reference when you collect beetles in successive years for the recovery report. CAUTION: Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on diffuse knapweed plants within a 3-4 foot radius of the stake.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 10:

Go to the next section--Fill Out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS) Fill out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data)

Introduction

These forms are used for recording the details of biocontrol agent shipments and releases. The information will be published by the Beneficial Insect Introduction Laboratory of the Agricultural Research Service, and you will be credited with the release. See an example of each form in Appendix 6.

Filling Out Forms

AD-943:

Blocks 1 through 18 will be completed for you.

Fill out Blocks 19 through 33 with the details of your release.

Block 19: Enter the date you received the shipment.

Block 20: Record the number and stages of biocontrol agents you received. Use the codes on the reverse of the form.

Block 21: Note the condition of the material upon its arrival. Examples might be, "Insects looked lively," "90 percent of insects dead," or "Insects alive but inactive."

Block 22: Record the number of specimens you retained for your voucher samples.

Block 23: Check box A (Immediate release).

Block 24: Enter "N/A" (not applicable).

Block 25: Check the "Field" box for each site. Use Section B of Form AD-943A if you have more than three release sites. **Do not use Section C.**

Block 26: Record the location of each site. Please provide township, range, and section information down to quarter section. For example, Sec. 32 SE is the southeast quarter of section 32. Record the latitude-longitude data as determined by the Transpak II GPS unit. Complete Section A, Form AD-943A. **NOTE:** If you have already provided this information on the FISPIS, it will NOT be necessary for you to do so again. Include a photocopy of a map of each release site or draw a map that relates the release site to some topographical feature.

Block 27: Record the number and stages of biocontrol agents you released. Use the codes on the reverse of the form.

Block 28: Enter the date you released the biocontrol agents.

Block 29: Enter Centaurea diffusa or Centaurea maculosa as the primary target host (line A.).

Block 30: Enter "N/A."

Block 31: Enter the name and affiliation of the actual releaser.

Block 32: Describe any special conditions at the time of the actual field release. This might include weather conditions (e.g., "Released in heavy rain, 45°F"), steps taken when you release the agents (e.g., "Release marked with blue-painted stake"), or cooperating personnel involved in, or present at, the release, especially if all their names do not fit in **Block 31**.

Block 33: Enter your name and date.

AD-943A:

Shipper's File Number: Copy this number from Block 3 of Form AD-943. Section A--

Township, route no., Farmer's name, etc. Map of release site.: Draw a map of the release site in the space provided.

WEATHER (including TEMP., WIND, SKY): Fill in these blocks using the example in Appendix 6 as a guide.

TIME OF RELEASE: Fill in this block using the example in Appendix 6 as a guide. CONDITION OF CROP FIELD: Fill in this block using the example in Appendix 6

CONDITION OF RELEASE MATERIAL: Fill in this block using the example in Appendix 6 as a guide.

Mailing Forms Return Forms AD-943 and AD-943A within 1 week of your release to BBCF (envelope enclosed). PLEASE REMEMBER TO APPLY POSTAGE!

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS) Collect Samples of Biocontrol Agents From the Phase 1 FIS

Introduction

Sampling is a tool that permits us to estimate the size of an insect population, and to describe some of the characteristics of that population. Reliable estimates of agent population size are essential in developing a sound redistribution strategy.

When to Collect Times to collect knapweed biocontrol agents vary greatly. The generalized dates are included in the text and instructions that follow.

Collecting Agaptea zoegana

Step 1:

Wait for a good day to collect the moths. The following conditions are ideal:

- Warm (45°- 50°F), dark evening; calm or with just a slight breeze.
- Dry vegetation. Plan to arrive around 9:30 to 10:00 p.m. The moths fly from 9:30 until 11:00 p.m.

Monitor from July 10 - August 10.

Step 2:

Prepare to collect the moths. Check to make sure you have the following items:

- Black ultraviolet (UV) light
- Three to four white bed sheets
- Small glass screw top vials
- Modified insect vacuum (see Appendix 8)
- Pencil(s)
- Post or stake
- Sledge hammer

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS (see Appendix 6).

Step 4:

Locate the metal stake that marks the exact release point.

<u>Step 5</u>:

Spend a minute or two looking over the site to note if you can readily see adult biocontrol agents on the knapweed plants.

Step 6:

Set up your black light on the hood of your vehicle or hang from a post or stake as close as possible to the release point.

Step 7:

Place a sheet on the hood of the vehicle near the light or suspend on stakes or shrubs. Lay two to three sheets flat on the ground at the inside edge of the light circle.

Step 8:

Walk just beyond the light circle and disturb the knapweed plants to scare into flight the *A. zoegana* moths.

Step 9:

Examine the sheets for moths, and count the moths every 15 minutes. Record the number of moths per 15 minutes.

Step 10:

Using the modified insect vacuum, collect two to three moths for voucher specimens. Kill them as soon as possible by freezing for 1 hour. Place them in a glass screw top vial for shipment or pin the specimens with a #2 insect pin.

Step 11:

Label the insect with release code, site name and date. Place the label on the pin with the insect. If you don't pin, place the label in the vial with the moths.

Step 12:

Mail your sample to BBCF with the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Step 13:

If you do not collect any samples, return only the completed Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Collecting **Bangasternus** and **Larinus** spp.

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Sunny, warm (>70°F) day; calm or with just a slight breeze.
- Dry vegetation. Plan to arrive at the release site NO EARLIER THAN 10 a.m. (heavy dew earlier in the morning may interfere with collection).

Plan to collect samples when the knapweed is at 1 percent bloom, again at 10 percent bloom, and again at 50 percent bloom.

Step 2:

Prepare to collect the seedhead weevils. Check to make sure you have the following items:

- 15-inch diameter sweep net
- 70 percent ethyl or isopropyl alcohol
- Small glass screw-cap vial
- Pencil(s)

<u>Step 3</u>:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS (see Appendix 6).

Step 4:

Locate the metal stake that marks the exact release point.

Step 5:

Spend a minute or two looking over the site to note if you can readily see adult *Bangasternus* or *Larinus* spp. weevils on the knapweed plants, especially in and on the flower heads.

Step 6:

Using the sweep net, collect samples at five points along four lines in N., S., E., and W. directions from the original release point.

- 1. For each line, begin as close as possible to the release point.
- 2. Make **four** sweeps in front of you (back and forth twice). Sweep the net vigorously in a downward arc through the vegetation.
 - 3. Carefully examine the net and count the weevils present.
 - 4. Empty the net to release the weevils you have counted.
- 5. Record the number of weevils you counted in the appropriate block on the back of the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT (see Appendix 6).
 - 6. Move two paces (5-6 ft.) out, and repeat the procedure.
- 7. Continue until five points have been sampled; then repeat over the remaining cardinal directions.

Step 7:

During the sweeping process collect a maximum of 15 weevils from the site and place them in 70 percent ethyl or isopropyl alcohol in a glass screw-cap vial.

Step 8:

Label the vial with the release code, site name, and date. Place the label inside the vial. CAUTION: Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 9:

Mail your samples to BBCF with the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Step 10:

If you do not collect any samples, return only the completed Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Collecting Cyphocleonus achates

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Sunny, warm (>70°F) day; Calm or with just a slight breeze.
- Dry vegetation. Plan to arrive at the release site NO EARLIER THAN 10 a.m. (heavy dew earlier in the morning may interfere with collection.

Plan to collect when the knapweed plants have reached full bloom with some ripe seedheads. You may find weevils up until a hard freeze.

Step 2:

Prepare to collect *Cyphocleonus achates*. Check to make sure you have the following items:

- 15-inch sweep net
- 70 percent ethyl or isopropyl alcohol
- Glass screw-cap vial
- Pencil(s)

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS (see Appendix 6).

Step 4:

Locate the metal stake that marks the exact release point.

Step 5:

Spend 10-15 minutes looking over the site to note if you can see adult *Cyphocleonus* achates weevils on the knapweed tops or down in the leaves of the knapweed rosettes.

Step 6:

Using the sweep net, collect samples at five points along four lines in N., S., E., and W. directions from the original release point.

- 1. For each line, begin as close as possible to the release point.
- 2. Make **four** sweeps in front of you (back and forth twice). Sweep the net vigorously in a downward arc through the vegetation.
 - 3. Carefully examine the net and count the weevils present.
 - 4. Empty the net to release the weevils you have counted.
- 5. Record the number of weevils you counted in the appropriate block on the back of the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT (see Appendix 6).
 - 6. Move two paces (5-6 ft.) out and repeat the procedure.
- 7. Continue until five points have been sampled; then repeat over the remaining cardinal directions.

<u>Step 7</u>:

During the sweeping process collect a maximum of three beetles from the site and place them in 70 percent ethyl or isopropyl alcohol in a glass screw-cap vial.

Step 8:

Label the vial with the release code, site name, and date. Place the label inside the vial. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

<u>Step 9</u>:

Mail your samples to BBCF with the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Step 10:

If you do not collect any samples, return only the completed Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Collecting
Chaetorellia,
Metzneria,
Terellia, and
Urophora spp.

Step 1:

Collect seedheads from March 1 - May 31.

<u>Step 2</u>:

Prepare to collect the fly or moth infested seedheads. Check to make sure you have the following items:

- Gallon size resealable plastic bags
- Pencil(s)

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS (see Appendix 6).

Step 4:

Locate the metal stake that marks the exact release point.

Step 5:

Collect seedheads in the following manner:

- 1. Collect two seedheads per plant along four lines in N, S, E, and W directions from the original release point.
 - 2. Collect 50 seedheads from each line for a total of 200 seedheads.
 - 3. As you collect the seedheads, place them in the resealable plastic bag.

Step 6:

Label the bag with release code, site name, and date. Place the label inside the resealable plastic bag, and seal the bag.

Step 7:

Mail your samples to BBCF with the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Collecting Sphenoptera jugoslavica

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Sunny, warm (>65°F) day; calm or with just a slight breeze.
- Dry vegetation. Plan to arrive at the release site NO EARLIER THAN 5 p.m.

Monitor when the diffuse knapweed plants are at about 1-10 percent bloom.

Step 2:

Prepare to collect the beetles. Check to make sure you have the following items:

- 15-inch diameter sweep net
- 70 percent ethyl or isopropyl alcohol
- Small glass screw-cap vial
- Pencil(s)

Step 3:

Drive to the Phase I FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS (see Appendix 6).

Step 4:

Locate the metal stake that marks the exact release point.

Step 5:

Spend a minute or two looking over the site to note if you can readily see adult *Sphenoptera jugoslavica* beetles on the diffuse knapweed plants.

Step 6:

Using the sweep net, collect samples at five points along four lines in N., S., E., and W. directions from the original release point.

- 1. For each line, begin as close as possible to the release point.
- 2. Make four sweeps in front of you (back and forth twice). Sweep the net vigorously through the vegetation in a downward arc, as close to the ground as possible.
 - 3. Carefully examine the net and count the S. jugoslavica beetles present.
 - 4. Empty the net to release the beetles you have counted.
- 5. Record the number of beetles you counted in the appropriate block on the back of the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT (see Appendix 6).
 - 6. Move 2 paces (5-7 ft.) out, and repeat the procedure.
- 7. Continue until five points have been sampled, then repeat over the remaining cardinal directions.

Step 7:

During the sweeping process, collect a maximum of 5 beetles from the site and place them in 70 percent ethyl or isopropyl alcohol in a glass screw-cap vial.

Step 8:

Label the vial with the release code, site name, and date. Place the label **inside** the vial. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 9:

Mail your samples to BBCF with the Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Step 10:

If you do not collect any samples, return only the completed Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS) Fill Out a Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT

Introduction

This form documents the results of your sampling efforts. It is very important to record your observations on this form because BBCF will use the data to estimate the population size of biocontrol agents. See Appendix 6 for an example.

Step 1

Record the information pertaining to location of the release site:

Release code: Enter the number provided to you by BBCF. BBCF assigns this code after receiving your FISPIS and Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

State and County: Record the state and county in which the release site is located.

Site name: Copy the site name from the appropriate FISPIS or Form AD-943.

USGS coordinates (United States Geological Survey): Provide township, range, and section information down to quarter section.

Latitude and longitude: Record the latitude-longitude data as determined by the Transpak II GPS unit. If you did not record these data at the time of initial release, do so now.

Step 2

Indicate which insect was released, the date of the original release, and whether the release was in a cage or in the open.

Step 3

Record the requested SAMPLING INFORMATION:

SAMPLING DATE and SAMPLING TIME (approx.): Enter the date (month, day, year), and the approximate local time you began sampling.

Weather conditions, Air temperature (F°), and Wind: Give your best estimations of these conditions. Ideally, measure the temperature with a thermometer.

Visual observation of insect, before sweeping: Just check for obvious presence of the insect. Do not spend more than 5 to 10 minutes looking.

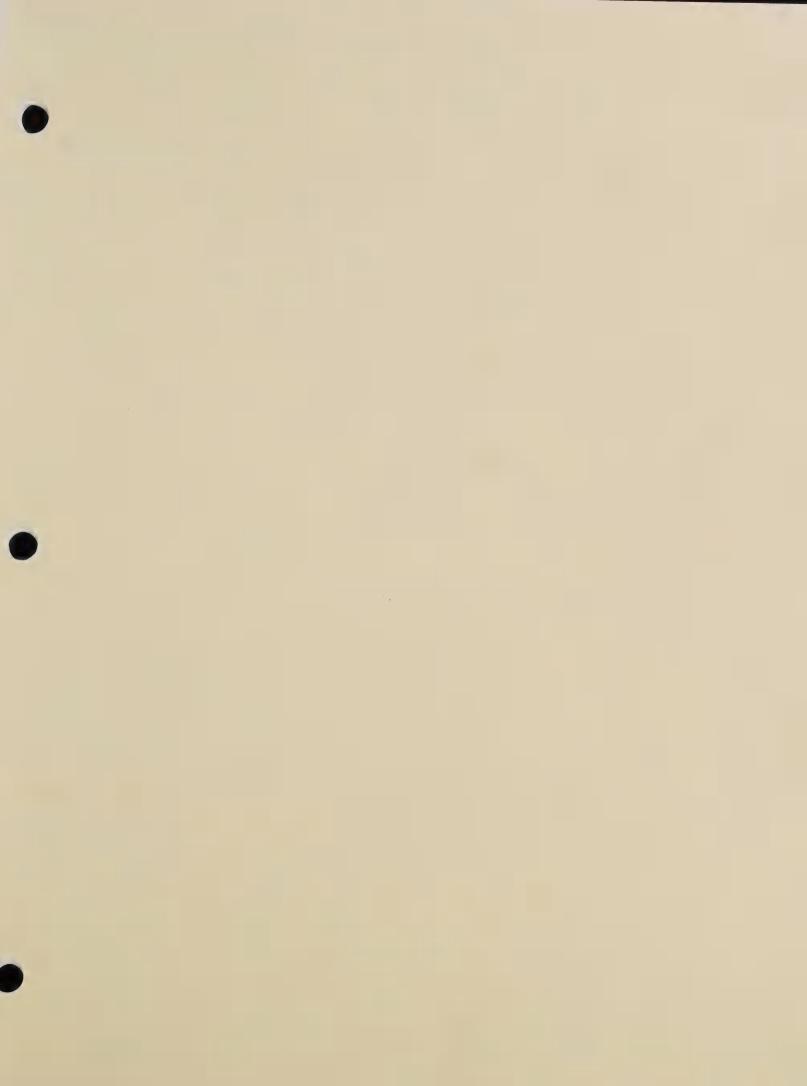
Number of Net sweeps: If you are sampling for *Bangasternus* spp., *Larinus* spp., or *Sphenoptera* spp., enter 80 (you should have made 4 sweeps at each of 5 sampling points along 4 lines).

For *Cyphocleonus achates*, number of adults observed: Record the number of adults you observed within the 50-75 ft. diameter survey area.

Observer, Affiliation, and Phone: Enter your name, organization, and phone number.

Step 4

See the reverse side of this form for the diagram of the sampling procedure and chart for recording insect counts. Record your insect counts in the appropriate blocks on the chart.





ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS)Introduction

When to Establish

The Bozeman Biological Control Facility (BBCF) will let you know when the time is right for establishing a Phase 2 Field Insectary in your State.

Purpose

Phase 2 Field Insectary Sites will serve two purposes:

- 1. To function as the source of additional natural enemies in 3 to 5 years for continued redistribution in Phase 3.
- 2. To serve as demonstration plots, showing the potential impact of the natural enemy on diffuse and spotted knapweeds.

Overview

If you are familiar with the process, you can use the following overview as a checklist of the tasks for establishing a Phase 2 Field Insectary.

- 1. Select a suitable site for the insectary.
- 2. Collect biocontrol agents from a Phase 1 FIS for redistribution to the Phase 2 FIS.
- 3. Release the biocontrol agents at the Phase 2 FIS.

Diffuse and Spotted Knapweed

ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS) Select a Suitable Site for the Insectary

Introduction

Establishing a Phase 2 FIS will be a cooperative effort by APHIS and State departments of agriculture and/or research cooperators. You must plan very carefully when you select a Phase 2 FIS, just as you did when you selected the Phase 1 FIS. BBCF will contact you to let you know when the time is right for establishing a Phase 2 FIS in your State.

Step 1

Contact State department of agriculture personnel and county extension educators. Tell them you have been informed by BBCF that your Phase 1 FIS has been successfully established, with enough biocontrol agents for redistribution.

Step 2

With input from State personnel who are knowledgeable about diffuse and spotted knapweed infestation, visit prospective sites to evaluate their suitability for establishing a Phase 2 FIS, using the following criteria:

- Stand density:
 - -- Moderately dense. Some bare ground is desirable.
- Sunlight:
 - -- Open fields without shade are best (the biocontrol agents like sun).
- Exposure:
 - -- Southern exposures are best. Neutral exposures (flat locations) are acceptable, while northern exposures are least desirable.
- Soil type:
 - -- Moderately textured loams are best. Clay soils are not as good.
 - -- The soil should be moderately to well drained, and the area should be free from seasonal flooding.
- Grazing:
 - -- The area should be free from grazing.
- Field size:
 - -- The field should be at least 2 acres large, preferably larger.
- Pesticide use:
 - -- The site should not be exposed to insecticides.
 - -- No herbicides should be used within 500 ft of the release point.
- Land ownership:
 - -- Public land is usually a better choice than private land, because of longevity of management.

- Step 3 After you have visited several prospective sites, choose a site that has most of the desirable characteristics listed above.
- Step 4 Contact the land owner or land manager in person or by telephone. Identify yourself (give a business card if available).
- Ask permission to establish a field insectary site. Make sure that the land owner or land manager is aware of, and willing to make the 5-year commitment described in item 25 on the Field Insectary Site Preliminary Information Sheet (FISPIS).

ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS)
Collect Biocontrol Agents From a Phase 1 FIS for Redistribution to a
Phase 2 FIS

Introduction

You must have a successfully established Phase 1 FIS in order to collect biocontrol agents for redistribution. Based on the data you provided on the Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT, BBCF will confirm that you have a large enough population from which to collect.

Collecting Agapeta zoegana

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Warm (45°-50°F), with a slight breeze.
- Dry vegetation. Plan to arrive around 9:30 to 10:00 p.m. (the moths fly from 9:30 until 11:00 p.m.).

Collect between July 10 and August 10.

Step 2:

Prepare to collect the moths. Check to make sure you have the following items:

- Black ultraviolet (UV) light
- Three to four white bed sheets
- Modified insect vacuum
- Shipping cartons (paper ice cream type)
- Paper towels
- Stakes or Posts
- Sledge hammer
- Cooler and blue ice pack
- Cardboard box for mailing insects
- Masking tape
- Pencil(s)

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS (see Appendix 6).

Step 4:

Collect A. zoegana moths.

- 1. Set up your black light on the hood of your vehicle or hang from a post or stake as close as possible to the release point.
- 2. Place a sheet on the hood of the vehicle near the light or suspend on stakes or shrubs. Lay two to three sheets flat on the ground at the inside edge of the light circle.
- 3. Walk just beyond the light circle and disturb the knapweed plants to scare into flight the A. zoegana moths.
- 4. Examine the sheets for moths, and vacuum them into the shipping carton from the sheets. Put no more than 50 moths in a quart carton with two crumpled paper towels in the carton.
- 5. Repeat the procedure until the moths are no longer coming to the sheets or you have the number desired.

<u>Step 5</u>:

Seal the carton(s) with masking tape. Do NOT punch holes in the carton.

Step 6:

Label the carton(s)--write on the lid the species, the number of moths you collected, and the date.

Step 7:

Place the shipping carton in the cooler with the blue ice pack. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the shipping carton on top of the foam beads or newspaper. DO NOT ALLOW THE SHIPPING CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 8:

Give the cooler containing the shipping carton to the State cooperator who will be working with the Phase 2 FIS, or mail the cooler in the cardboard box to the cooperator, if necessary. THE COOPERATOR MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Collecting Bangasternus spp. and Larinus spp.

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Sunny, warm ($>70^{\circ}$ F) day; calm or with just a slight breeze.
- Dry vegetation. Plan to arrive at the release site **NO EARLIER THAN 10 a. m.** (heavy dew earlier in the morning may interfere with collection).

Plan to collect when the knapweed is at 1 percent to 10 percent bloom.

Step 2:

Prepare to collect the seedhead weevils. Check to make sure you have the following items:

- 15-inch diameter sweep net
- Gallon shipping cartons (paper ice cream type)
- Cooler and blue ice pack
- Clippers for collecting knapweed shoot tips
- Masking tape
- Pencil(s)

<u>Step 3</u>:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS (see Appendix 6).

<u>Step 4</u>:

Use your clippers to collect knapweed shoot tips that DO NOT HAVE FLOWERS OR SEEDHEADS. Do NOT include any flowers or seedheads with the shoot tips. Collect enough shoot tips to fill the carton one-fourth full. Place the tips in the carton.

Step 5:

Using the net, sweep vigorously through the vegetation to collect as many beetles as possible.

Step 6:

Dump the contents of the net into a gallon carton (cardboard ice-cream type). You may need to shake the contents from the net into the carton. Do not dump more net fulls than 10 times per gallon carton.

Step 7:

Seal the carton and place it in the cooler with blue ice. DO NOT LET THE CARTON TOUCH THE BLUE ICE.

Step 8:

Repeat this process for additional cartons until you have the number of weevils you want.

Step 9:

Choose a method to sort the weevils:

Method I

- 1. Sift the trash, the insects, and weevils through a series of graduated screens in the following sequence: #10 (2.0 mm), #14 (1.4 mm), #18 (1.0 mm), and #25 (710 μ m). This will eliminate the knapweed seed from the weevils.
- 2. Once the weevils have been separated from the knapweed seed, pick out the larger pieces of trash, pick off the weevils, and place in the carton.
- 3. Use life tweezers to pick up the remaining weevils and put them in the carton.

Method II

- 1. Place a handful of trash in a flat pan--let the weevils leave the trash, pick them up with life tweezers, and place them in the carton.
- 2. With the tweezers work through part of the trash at a time until all the trash has been sorted. Look for weevils, collect, count, and place them in the carton.

DO NOT PUT MORE THAN 500 WEEVILS IN A CARTON. Put some knapweed shoot material in the carton with the weevils.

Step 10:

Seal the cartons with masking tape. DO NOT punch holes in the carton(s).

Step 11:

Label the carton(s)-- write on the lid the species, the number of weevils in the carton, and the date.

Step 12:

Place the shipping carton in the cooler with the blue ice pack. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the shipping carton on top of the foam beads or newspaper. DO NOT ALLOW THE SHIPPING CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

<u>Step 13</u>:

Give the cooler containing the shipping carton to the state cooperator who will be working with the Phase 2 FIS, or mail the cooler in the cardboard box to the cooperator, if necessary. THE COOPERATOR MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Collecting Cyphocleonus achates

<u>Step 1</u>:

Wait for a good day to collect the biocontrol agents. A sunny, warm (>70°F) day is ideal; calm or with just a slight breeze. Plan to arrive at the release site **NO EARLIER THAN 10 a. m.** (The weevils like the heat of the day.)

Collect when the knapweed is in full bloom and some ripe seedheads are present.

Step 2:

Prepare to collect *Cyphocleonus achates* weevils. Check to make sure you have the following items:

- Shipping carton (paper ice cream type)
- Cooler and blue ice pack
- Clippers for collecting knapweed shoot tips
- Masking tape
- Pencil(s)

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of the photocopy of the FISPIS (see Appendix 6).

<u>Step 4</u>:

Use your clippers to collect knapweed shoot tips that **DO NOT HAVE FLOWERS OR SEEDHEADS.** Do **NOT** include any flowers or seedheads with the shoot tips. Collect enough shoot tips to fill the carton one-fourth full. Place the tips in the carton.

Step 5:

Examine the knapweed plants carefully to find the weevils. *Cyphocleonus achates* weevils tend to climb to the tops of the plants. You can pick the weevils off the seedheads and flowers. After you have exhausted this method, carefully part the vegetation at the ground level, and look for weevils among the leaves and stems. You will need to look closely; the weevils are well camouflaged.

Step 6:

As you pick the weevils off the plant, place them in the shipping carton and put on the lid to keep the weevils from escaping. Place NO MORE THAN 50 WEEVILS in a quart carton.

Step 7:

Seal the carton and place it in the cooler with blue ice. DO NOT LET THE CARTON TOUCH THE BLUE ICE.

Step 8:

Repeat this process for additional cartons until you have the number of weevils you want.

Step 9:

Seal the cartons with masking tape. Do NOT punch holes in the cartons.

Step 10:

Label the carton(s)--write on the lid the species, the number of weevils in the carton, and the date.

Step 11:

Place the shipping carton in the cooler with the blue ice pack. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the shipping carton on top of the foam beads or newspaper. DO NOT ALLOW THE SHIPPING CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 12:

Give the cooler containing the shipping carton to the state cooperator who will be working with the Phase 2 FIS, or mail the cooler in the cardboard box to the cooperator, if necessary. THE COOPERATOR MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Collecting
Chaetorellia,
Metzneria,
Terellia, and
Urophora spp.

Step 1:

Wait for a good day. Dry vegetation will be easier to collect into bouquets. Collect from March 1 - May 15.

Step 2:

Prepare to collect the seedhead agents (the insects are larvae in the seedheads and will emerge as adults at the proper time). Check to make sure you have the following items:

- Wide rubber bands
- Heavy-duty gloves

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of the photocopy of the FISPIS (see Appendix 6).

Step 4:

Break off last year's knapweed plants, and develop bouquets as large in diameter as your hand will hold.

Step 5:

Place a wide rubber band on the stem part of each bouquet to hold it together.

Step 6:

Stack the bouquets and repeat Steps 4 and 5 until you have enough bouquets for the desired releases. Estimate the number of *Urophora* flies per bouquet using the following formula:

Estimated number of seedheads per bouquet (800 seedheads) x the number of *Urophora* fly galls per seedhead = the number of *Urophora* flies per bouquet. Example: 800 seedheads x an average of two *Urophora* fly galls per seedhead = 1600 *Urophora* flies per bouquet. Ten bouquets make one release.

Step 7:

Load the bouquets into a vehicle with a tarp or other barrier to prevent seed spread.

Step 8:

Drive to the new location for redistribution. Unload the bouquets and secure one bouquet per post or stake.

An alternative method for collecting seedhead agents is to strip seedheads from last year's plants. The seedheads are collected between March 1 and May 15 and placed in a 10-foot x 10-foot cage on double screen racks. The adult seedhead agents emerge and are collected from the cage sides with an insect vacuum.

If you plan to use this method, you will need the following equipment for rearing the seedhead agents:

- Double layer screen racks 4-foot x 8-foot
- 10-foot x 10-foot cage
- Portable insect vacuum
- Heavy duty 12-volt battery

<u>Step 1</u>:

Wait for a dry day. Plan to arrive at the site no earlier than 10 a.m. (heavy dew earlier in the morning will interfere with collection of seedheads).

Step 2:

Prepare to collect the seedheads. Check to make sure you have the following items:

- Heavy-duty gloves
- Heavy-duty 30-gallon plastic garbage bags

Step 3:

Strip seedheads from last year's plants, and place them in the garbage bags.

Step 4:

Transport the seedheads to your cage rearing site.

Step 5:

Put the seedheads one bag per 4-foot x 4-foot section of screen rack. A 10-foot x 10-foot cage will have space for 2 double layer 8-foot x 8-foot screen racks. You can put 8 bags of seedheads in a cage.

<u>Step 6</u>:

The flies will begin to emerge about the same time the knapweed begins to bolt and form buds. When the flies begin to emerge, collect daily with your portable insect vacuum. Collect 500 flies per tube by moving the nose of the vacuum back and forth across the cage side. When collecting *Metzneria* use the modified insect vacuum, and collect 50 moths per tube.

Step 7:

Keep the tubes filled with seedhead agents in a cooler with a blue ice pack.

DO NOT LET THE TUBES COME IN CONTACT WITH THE BLUE ICE.

Step 8:

When you have finished collecting, transport your cooler with tubes, and place the tubes in a refrigerator at 40°F for 15 to 20 minutes.

Step 9:

Remove one tube at a time from the refrigerator and take the cap off the tube. Put some wood excelsior or shredded paper in each carton before putting in the seedhead agents. Pour the seedhead agents into a quart carton (ice-cream type). DO NOT PUT MORE THAN 100 FLIES OR 50 MOTHS IN EACH CARTON. Put the top on securely.

Step 10:

Seal the carton with masking tape. DO NOT PUNCH HOLES IN THE CARTON!

Step 11:

Label the carton(s)--write on the lid the species, the number of seedhead agents you collected, and the date.

Step 12:

Place the shipping carton in the cooler with the blue ice pack. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the shipping carton on top of the foam beads or newspaper. DO NOT ALLOW THE SHIPPING CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 13:

Give the cooler containing the shipping carton to the state cooperator who will be working with the Phase 2 FIS, or mail the cooler in the cardboard box to the cooperator, if necessary. THE COOPERATOR MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Collecting Sphenoptera jugoslavica

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Sunny, warm (>65°F) day; calm or with just a slight breeze.
- Dry vegetation. Plan to arrive at the release site NO EARLIER THAN 5 p.m.

<u>Step 2</u>:

Prepare to collect the beetles. Check to make sure you have the following items:

- 15-inch sweep net
- Shipping cartons (paper ice-cream type)
- Cooler and blue ice pack
- Clippers for collecting knapweed shoots
- Cardboard box for mailing insects
- Masking tape
- Pencil(s)
- Cloth bag or pillowcase

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of the photocopy of the FISPIS (see Appendix 6).

<u>Step 4</u>:

Use your clippers to collect knapweed shoots that DO NOT HAVE FLOWERS OR SEEDHEADS. Do NOT include any flowers, seeds, or roots with the shoot tips. Collect enough shoot tips to make the carton one-fourth full. Place the shoots in the carton.

Step 5:

Using the net, sweep vigorously through the vegetation to collect as many beetles as possible.

Step 6:

Empty the net, approximately every 20 sweeps, into a bag folded over your belt. Turn the net inside out and shake into the bag all the material sticking to the sides of the net into the bag.

Step 7:

Keep the accumulation cartons in a cooler with blue ice packs while you continue to collect. **DO NOT LET THE CARTON TOUCH THE BLUE ICE.** Refrigerate the collected material overnight when possible.

Step 8:

The beetles need to be sorted from seed, other insects, and trash. Do this the next morning. Place a handful of trash and beetles in a flat pan. The beetles will crawl out to the sides across the pan. Pick up the beetles with a pair of life tweezers and put them into a carton with some knapweed shoots. DO NOT PUT MORE THAN 200 BEETLES IN A QUART CARTON.

Step 9:

Seal the quart cartons with masking tape. Do NOT punch holes in the carton.

Step 10:

Label the carton(s)--write on the lid the species, the number of beetles you collected, and the date.

Step 11:

Place the shipping carton in the cooler with the blue ice pack. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the shipping carton on top of the foam beads or newspaper. DO NOT ALLOW THE SHIPPING CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!



Give the cooler containing the shipping carton to the State cooperator who will be working with the Phase 2 FIS, or mail the cooler in the cardboard box to the cooperator, if necessary. THE COOPERATOR MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS) Release the Biocontrol Agents at a Phase 2 FIS

Introduction

RELEASING BIOCONTROL AGENTS AT A PHASE 2 FIS IS A COOPERATOR ACTIVITY. The steps for releasing biocontrol agents at a Phase 2 FIS are the same as the steps for releasing biocontrol agents at a Phase 1 FIS. You will not, however, release *Pterolonche inspersa* and *Pelochrista medullana* at a Phase 2 FIS in FY 95.

Releasing Agapeta zoegana

Step 1:

After you receive the moths from the collector, open the shipping package and place in a refrigerator the carton containing the biocontrol agents. (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THIS BIOCONTROL AGENT THE SAME DAY YOU RECEIVE IT.

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pace in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the moths are alive, sacrifice and retain two or three for your voucher sample.

Step 6:

Release the biocontrol agents on spotted knapweed within a 3-foot radius of the stake.

<u>Step 7</u>:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 8:

Pin the moths you saved immediately upon return to your office.

Step 9:

Place a label on the pin with the insect so you will have these specimens as a reference.

Releasing Bangasternus fausti and Larinus spp.

Step 1:

After you receive the seedhead weevils from the collector, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by driving a metal stake securely into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the beetles are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the beetles you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Label the vial so you will have these specimens as a reference. CAUTION: Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on spotted or diffuse knapweed (except L. obtusus: spotted only) within a 3-foot radius of the stake.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Releasing Cyphocleonus achates

Step 1:

After you receive the weevils from the collector, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

<u>Step 3</u>:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the weevils are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the weevils you retained in a glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Label the vial so you will have these specimens as a reference. CAUTION: Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on spotted or diffuse knapweed plants within a 3-foot radius of the stake. Take time to hand place three to four weevils per plant.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Releasing Chaetorellia acrolophi, Terellia virens, and Urophora spp.

Step 1:

After you receive the seedhead flies from the collector, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather.

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the flies are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the flies you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Label the vial so you will have these specimens as a reference. CAUTION: Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on spotted and diffuse knapweed plants within a 3-foot radius of the stake.

Step 9:

Return the empty carto to your vehicle (do not leave the carton at the release site).

Releasing Metzneria paucipunctella

Step 1:

After you receive the moths from the collector, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather!

Step 4:

Mark the release point by securely driving a metal stake into the ground.

<u>Step 5</u>:

Count and retain dead biocontrol agents for voucher samples. If all of the moths are alive, sacrifice and retain two or three for your voucher sample.

Step 6:

Release the biocontrol agents on spotted knapweed within a 3-foot radius of the stake.

Step 7:

Return the empty carton to your vehicle (do not leave the carton at the release site).

Step 8:

Pin the moths you saved as soon as you return to your office.

Step 9:

Place a label on the pin with the moth so you will have these specimens as a reference.

Releasing Sphenoptera jugoslavica

Step 1:

After you receive the beetles from the collector, open the shipping package and place the carton containing the biocontrol agents in a refrigerator (NOT FREEZER!) at 40°-50°F until you are ready to transport the agents to the field. YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!

Step 3:

Drive to the release site. DO NOT wait for good weather.

Step 4:

Mark the release point by securely driving a metal stake into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the beetles are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the beetles you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Label the vial so you will have these specimens as a reference. **CAUTION**; Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on diffuse knapweed plants within a 3-4 foot radius of the stake.

Step 9:

Return the empty carton to your vehicle (do not leave the carton at the release site).





APPENDIX 1: DIFFUSE AND SPOTTED KNAPWEED PLANTS

Introduction

Use this appendix to help identify diffuse and spotted knapweed plants and infested rangeland. The number in brackets [] corresponds to the photograph number. The photographs are located in the back of this appendix.

Photographs of Knapweed Plants

Centaurea maculosa (Spotted Knapweed) [1-1], [1-2]:

Spotted knapweed is a biennial or short-lived perennial herb with a stout tap root. The purple or white flower heads are terminal and axillary. The brown spots on the seedhead bracts are characteristic of this plant. Spotted knapweed branching tends to be open and airy.

Spotted Knapweed Infested Range [1-3]:

Spotted knapweed was introduced from Eurasia as a contaminant of alfalfa and clover seed. Spotted knapweed competitiveness reduces desirable diverse plant communities. Spotted knapweed readily establishes on any disturbed soil and is very competitive for soil moisture and nutrients.

Spotted Knapweed Rosette [1-4]:

Spotted knapweed rosettes are the choice feeding and oviposition sites of the root boring biocontrol agents. The plant will remain as a rosette until there is enough moisture and favorable climatic conditions to cause bolting.

Spotted Knapweed Mature Dry Seedheads [1-5]:

The dry seedheads and dead plants remain in the field 1-2 years. New growth starts each year form the plant crown. Some spotted knapweed biocontrol seedhead agents overwinter in the seedheads.

Centaurea diffusa (Diffuse Knapweed) [1-6], [1-7]:

Diffuse knapweed is a biennial herb with an elongated taproot. The flowering heads are numerous and narrow. The ray flowers are usually white. Purple flowers may be found.

Diffuse Knapweed Shape and Form of Branching [1-8]:

Diffuse knapweed plants are often bushy and thick. Wind breaks off the dry mature knapweed plant, and seed is dispersed as the plant is blown in the wind.

<u>Diffuse Knapweed Infested Range [1-9]:</u>

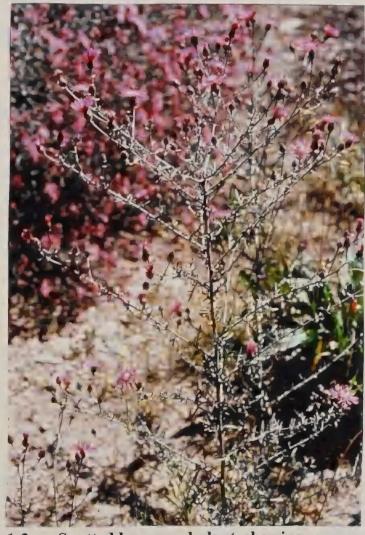
Diffuse knapweed may be found along roads, waste areas, and dry rangelands. It is a highly competitive plant that threatens to exclude many desirable forage species.



1-1 Centaurea maculosa Lam. (spotted knapweed) flower head



1-3 Spotted knapweed infested range



1-2 Spotted knapweed plant, showing branching form



1-4 Spotted knapweed rosette



1-5 Mature spotted knapweed seedheads



1-6 Centaurea diffusa Lam. (diffuse knapweed) uncommon purple flower head



1-7 Diffuse knapweed normal white flower head



1-8 Diffuse knapweed plant showing branching and form



1-9 Diffuse knapweed infested range





APPENDIX 2: BIOLOGY AND IDENTIFICATION OF BIOCONTROL AGENTS

Introduction

Use this appendix to help identify the biocontrol agents of diffuse and spotted knapweeds. The appendix includes photographs as well as narrative descriptions of these biocontrol agents. The number in brackets [] corresponds to the photograph number. The photographs are located in the back of this appendix.

Agapeta zoegana

Life Cycle:

Agapeta zoegana, a root boring moth, undergoes complete metamorphosis and has one generation per year. The adult moths emerge from July through September, with the males emerging first. Because emergence is temperature dependent, warmer areas can expect adult moths earlier in the season.

Females mate within 24 hours of emergence and begin egg laying the next day. Temperatures of 18°C (30°F) to 65°C (86°F) are needed for mating, with optimal egg laying at 30°C (86°F). The minimal temperatures tolerated for egg laying are 20°-24°C (68°-75.5°F). The females that emerge in the middle of the season lay the greatest numbers of eggs.

Eggs are laid singly or in small batches in crevices on the surface of the leaves and stems. The females prefer to lay their eggs on the lower leaves of the knapweed plant.

The larvae hatch in 7-10 days, migrate into the root crown and mine just below the outer surface of the main root. They destroy the vascular system and may completely destroy small roots. In dense stands, larvae may move from one plant to another. The larvae overwinter in the root crown, then feed and pupate in the spring. The moth's effect on the plants is death of rosettes, and stunting and stressing of older plants.

Identification:

Adults: A. zoegana moths are 1-2 cm long [2-1]. The bright yellow wings with brown bands resemble a dead or dying knapweed leaf. The adults rest during the day on the stems or the underside of the leaves of knapweed plants. Flight occurs during the evening hours from approximately 9 p.m. to 10:30 p.m. The moths may fly upward if disturbed during the day, but generally flutter to the ground and sit still, blending with the duff and dead leaves.

Larvae: The larvae [2-2] are white, with a distinct brown head capsule and six distinct legs. They are found in the upper part and just below the outer surface of the root.

Bangasternus fausti

Life Cycle:

Bangasternus fausti weevils have one generation per year and feed on the seedheads of diffuse and spotted knapweeds. The adults emerge from August to September and overwinter in debris and soil or in the seedheads of the knapweed. The larvae develop in the seedhead.

The adult female lays one egg in the flower bud where she has eaten some of the light hairs in preparation for egg laying. After the egg is laid it is covered with a dark green substance exuded from the anus. The substance hardens in 2-3 minutes and becomes black in color. The females sometimes lay eggs on the bracts of the flower head, the terminal portion of the stem, or the flower stalk. Each female may lay up to 300 eggs.

The newly hatched larvae mine into the flower bud, and by the time the larvae mature, they have destroyed nearly the entire contents of that bud. Any seeds that remain fail to germinate.

Identification:

Adults: The adults are small, dark brown to grey weevils [2-9]. They are most active on warm days, and may be found feeding on the open flowers of the knapweed.

Larvae and Pupae: The larvae are white grubs with a distinct head capsule. The pupae are white, turning brown just before emergence.

Chaetorellia acrolophi

Life Cycle:

Chaetorellia acrolophi, a seedhead fly, undergoes complete metamorphosis, following the sequence egg \rightarrow larva \rightarrow pupa \rightarrow adult. There are usually two generations per year. The emergence of adults starts in June and continues into the fall, with the two generations overlapping in July.

The second generation overwinters in the seedheads. The males and females emerge simultaneously and begin mating immediately.

The eggs are laid singly or in small batches underneath the bracts of closed buds, resulting in multiple larvae in a seedhead. A single female may lay up to 70 eggs with 1-12 eggs per seedhead.

The larvae hatch in 4-5 days at 20°C (68°F). They burrow into the immature florets and feed there for two instars. The older larvae mine into and feed on the developing seeds and receptacle of the flower head. The result of larval feeding is almost complete destruction of the seedhead contents. Up to 92.3 percent of the seeds in the attacked heads can be destroyed. The second generation larvae overwinter in the seedhead and pupate in the spring. *C. acrolophi, Urophora affinis*, and *U. quadrifasciata* may coexist in the same seedhead. Adults may be seen feeding on the immature knapweed flower buds throughout the season. Adults may live up to 30 days.

Identification:

Adults: Adults are dark bodied, with yellow bands on the body and wings [2-16].

Larvae: The first and second generation larvae and pupae differ in color: The first generation is white; the second generation is yellow. The pupae of both generations are enclosed in a pupal case covered with pappus hairs from the seeds.

Cyphocleonus achates

Life Cycle:

Cyphocleonus achates is a root boring weevil that undergoes complete metamorphosis and produces one generation per year. Adult emergence is from late July into October, depending on the accumulative soil temperature. The adult weevils live from 8-15 weeks, until the temperature drops below freezing.

Mating takes place soon after the adults emerge. Multiple mating takes place throughout the egg laying season. The female lays an average of 45 eggs and has the potential to lay 100 eggs. The female lays one egg at a time below the surface of the ground next to the root of the knapweed plant. She digs a hole next to the plant and bites a hole in the exposed root for an egg laying site. She leaves the hole, then backs into the hole, and lays the egg. The female leaves the hole again, turns around and reenters it head first, covering the egg with frass and plant material. After backing out of the hole, she then fills it with dirt. The entire process takes about 30 minutes.

The larvae hatch in 10-12 days at a temperature of 25°C (77°F), mine into the root crown and begin feeding. The larvae normally mine the root up to 8 cm below the root collar. They overwinter in the root and feed again in the spring, pupating in a gall-like chamber in the root.

C. achates adults are most active at temperatures above 20°C (68°F). Adults may be found on top of the plants during the heat of the day. The adults feed heavily on the knapweed plants throughout the mating season.

Identification:

Adults: Adult *C. achates* are weevils that measure \(^{3}\)-\(^{3}\) inch long [2-4]. The nose is broad and prominent. The color may vary from mottled sand to a dark mottled gray. The body is a pointed cylinder. The adult weevils do not fly.

Larvae: The larvae are large white grubs with a dark head capsule and six distinct legs. Multiple larvae may be found in the same root. The larvae negatively affect the nutrient flow into the stem.

Larinus minutus

Life Cycle:

Larinus minutus, a seedhead weevil, undergoes complete metamorphosis and produces one generation a year. L. minutus attacks both diffuse and spotted knapweed. The adults emerge from September through October, feed, and then hibernate in the duff and debris on the ground. They emerge from the trash in the spring, feeding on the knapweed foliage before bud development occurs. Adult weevils may live 5-14 weeks after spring emergence.

Mating takes place on the developing knapweed buds, followed by egg laying in fully opened flower buds. The females must feed on the flowers for ovary development. The female feeds on the florets around the mid-region of the flower head, preparing a site to deposit the egg. The egg is laid deep into the flower head, between the pappus hairs. The eggs develop in 3 days at 25°C. Complete larval development takes place in around 4 weeks. The larvae emerge, feed on the pappus hairs, and work their way to the achenes, consuming the entire contents of the individual seeds. Heavy feeding may result in the receptacle being attacked.

A single L. minutus larva may develop in the seedhead of diffuse knapweed. In spotted knapweed, several larvae may complete development in the same seedhead.

Identification:

Adults: Adult weevils are 5-10 mm long and mottled-brown in color [2-6].

Larvae and Pupae: The larvae are white grubs with a distinct head capsule. The pupae are white, turning brown just before emergence. The pupae will be in a cocoon covered with seed hulls [2-7].

Larinus obtusus

Life Cycle:

Larinus obtusus weevils undergo complete metamorphosisis and produce one generation a year. The larvae attack and develop in the seedheads of spotted knapweed.

The adults appear after overwintering in the debris in late spring, and begin to mate and lay eggs when the knapweed begins to flower. A female may lay from 21-81 eggs.

The eggs are laid in open flowers. Upon hatching, the larvae work their way into the flower, and during their development, destroy nearly all the seeds in the seedhead. The larvae develop from egg to adult in approximately 4-6 weeks.

Multiple larvae may develop in a seedhead. Each larva, as it matures, forms a cavity in the flowerhead and secretes a substance that hardens into a cocoon-like structure. The mature adult chews a hole in the center of the flower and emerges, feeds on the plants, and then moves into the soil and litter to overwinter. Adults have been observed to hibernate two winters and live a second year.

Identification:

Adults: The adult weevils are dark, and larger than *Larinus minutus* [2-8]. They have long, broad noses, flared at the nostril, which are characteristic of the genus *Larinus*. They are approximately 0.63 cm or 1/4 inch long.

Larvae and Pupae: The larvae are white grubs with a distinct head capsule. The pupae are white, turning brown just before emergence.

Metzneria paucipunctella

Life Cycle:

Metzneria paucipunctella, a seedhead moth, undergoes complete metamorphosis and produces one generation per year. The larvae overwinter in the seedhead, with the adults emerging from June through July. Jim Story, Montana State University, has found that the larvae cannot tolerate temperatures below -22°F. Therefore, this agent is not recommended for areas that experience temperatures below that limit. Adult moths begin mating soon after emergence, and egg laying takes place within 3 days of emergence.

During a three-week period, adult females lay eggs at the base of, or on the stem just below the closed flower buds. The larvae hatch in 10-12 days. They climb to the now open flower, and penetrate into the interior. The larvae feed on the seeds, and in the late instars, attack the receptacle. The larvae commence feeding again in the spring after winter hibernation, and then pupate. The adults fly at dusk and are rarely seen.

Identification:

Adults: The adult moth is pale brown, 1 cm long, and has a wing span of approximately 15 mm [2-10].

Larvae: The larva is a white grub with a distinct head capsule and six true legs. The contents of the seedhead form a chamber below the receptacle [2-11].

Pelochrista medullana

Life Cycle:

Pelochrista medullana is a root boring moth. It produces one generation per year and undergoes complete metamorphosis. The males and females emerge simultaneously in June and July from the roots of diffuse or spotted knapweed.

The moths mate within 24 hours of emergence in temperatures ranging from 18°-30°C. Egg laying begins within 2-3 days after emergence and continues for an average of up to 10 days. The eggs are laid singly or in small batches of up to three eggs, usually on the lower leaves of rosettes.

Temperature strongly influences egg laying. Low temperatures during the pre-pupal and pupal stages directly affect the breeding success of the adults and ultimately the hatching potential of the eggs. The mean temperature for successful reproduction must remain above 18°C during a 3-4 week period in the summer. Ideal temperatures, during warm, dry weather, are 15°-30°C.

The larvae hatch in 7-9 days. They immediately move to the center of the rosette where they mine into the root crown. Young larvae damage the cortical tissue just below the exodermis. The older larvae mine progressively downward in an open irregular, sometimes spiral mine. A whitish web covers the spiral mine. The larvae start a period of rest in October and resume feeding in the spring of the following year. They then pupate and in late June through July emerge as adults.

This moth prefers moist areas and will develop only on plants in the rosette stage. Diffuse knapweed is the preferred host plant.

Identification:

Adults: Adults are gray mottled-brown moths measuring 14-21 mm long.

Pterolonche inspersa

Life Cycle:

Pterolonche inspersa is a root boring moth. It has one generation per year and undergoes complete metamorphosis. The adults emerge from mid June to the end of July, and mate within 24 hours of emergence. Egg laying begins within 2-3 days after mating and lasts approximately 3 weeks.

Eggs hatch in about 10-12 days in temperatures that fluctuate between 15°C and 30°C. The newly hatched larvae immediately enter the plant tissue, complete their development in the root, and emerge as adults the following spring.

Up to four larvae feed in the root through the fall, overwinter in a silky cocoon on the root, and resume development and pupate in the spring. Pupation takes place in late spring.

P. inspersa prefers diffuse knapweed and thrives in hot, dry habitats with low to moderate densities of diffuse knapweed.

Identification:

Adults: The adults are grey-white and measure 14-28 mm long [2-3].

Larvae: The larvae are white with a distinct head capsule.

Sphenoptera jugoslavica

Life Cycle:

Sphenoptera jugoslavica is a root boring beetle that undergoes complete metamorphosis. The larvae burrow into the roots of diffuse knapweed, while the adults live up to 30 days, feeding on the leaves of the plant.

There is broad variation in the pattern of emergence, larval development, and egg laying, which reflect the diffuse knapweed rosette development. S. jugoslavica produces one generation per year.

The larvae overwinter in galled roots, feed again in the spring of the following year, pupate, and emerge as adults. Adult emergence begins when the knapweed is 1 percent in bloom. The adults mate 3 days after emergence and lay eggs in 5-12 days after mating. The female lays eggs between the bracts of the rosette leaves, in the leaf axil, or in the cleft between the buds and the main shoot. A female can be expected to lay an average of 47 eggs.

The newly hatched larvae feed on the peripheral tissues of the petiole base, but do not enter the tissue until after the first molt. A root gall begins to form when the larvae mine into the root. The gall formation takes place between July and September.

Before the larvae pupate, they tunnel to the root crown and form a pupal chamber [2-19]. The pupae are white for the first half of their development and turn black in the second half. The newly formed adults remain motionless in the root gall for 2-5 days. When they are ready to emerge, the adults bite a hole in the root and exit.

The effect of *S. jugoslavica* is that the plants are usually stunted, tensile strength is reduced, the plants break off easily, and some rosettes may not bolt. The unbolted knapweed rosettes are acceptable for secondary egg laying. Upon hatching, these larvae mine above or below the old gall.

Identification:

Adults: The adults are dark copper to black, elongated, 7-10 cm long beetles [2-18]. They appear flat. Adults will drop to the ground if disturbed, although they will tend to fly from the containers or sweep net.

Larvae and Pupae: The larvae are white, somewhat flattened with the anterior one-fifth enlarged with a distinct head capsule. The pupae are white, turning black in the last half of development.

Terellia virens

Life Cycle:

Terellia virens, a seedhead fly, undergoes complete metamorphosis, and usually produces two generations per year. The first and second generations may overlap in July, depending on the temperature. Adults emerge from June through late August; the first generation ends in July as the second generation begins to emerge. Emergence may begin up to 4 weeks before the knapweed seedheads are mature enough for the females to lay eggs. Adult flies live up to 48 days and feed heavily on knapweed flowers.

Adult females each lay an average of 80 eggs in young, opening knapweed flowers. Inserting the ovipositor from above into the flowerhead, they place the egg between the florets. The larvae hatch within 3-5 days at 20°-22°C (68°-72°F). Feeding on the florets and eventually entering the developing seed, the larvae complete development and pupate in about 14 days. As the larvae reach the end of the second instar, they emerge from that seed and feed on other seeds in the head. Larval feeding on older and harder seeds damages the seeds. The larvae of the second generation hibernate as prepupae in the fall and pupate in the spring.

Although *Urophora affinis* and *T. virens* are often found sharing the same seedhead, *T. virens* does not do well where *Larinus minutus* are abundant. It is best to avoid releasing *L. minutus* and *T. virens* at the same site.

Identification:

Adults: Adult T. virens are clear-winged, with a yellow-greenish body [2-17].

Larvae and Pupae: All stages of Terellia virens larvae and pupa are yellow-brown.

Urophora affinis

Life Cycle:

Urophora affinis, a seedhead fly, may give rise to two generations per year. The majority of the larvae will become dormant and emerge in early June to the end of July. U. affinis undergoes complete metamorphosis.

The males will guard and defend a plant as their territory. The females fly from plant to plant, mating on the flower buds with the guarding males. Multiple matings will take place during the 3-4 week life span of *U. affinis* adults. Egg laying begins 3 days after mating.

A female *U. affinis* may lay an average of 5-10 eggs a day with a potential of 120 eggs over a 3-4 week period. The female deposits eggs singly or in a mass on closed knapweed buds. She may probe the seedhead for suitable egg laying sites for up to an hour.

The larvae hatch and mine into the tubular part of the flower, penetrating into the flower head. Here the mining of the larvae causes the plant to form a hard, pointed gall [2-13]. It is possible to get up to 15 galls in a spotted knapweed seedhead. The larvae damage the plant by destroying the achenes and deforming the receptacle, reducing production of viable seed.

Identification:

Adults: Adult *U. affinis* flies are 1-3 mm long, with dark bodies and clear wings that have faintly marked bars. Females have a prominent dark ovipositor [2-12].

Larvae and Pupae: Larvae are white with a brown base plate. The pupae are clear rust brown, darkening and becoming opaque as they mature.

Urophora quadrifasciata

Life Cycle:

Urophora quadrifasciata undergoes complete metamorphosis and has two generations each year. The overwintering adults emerge in late June through July, as the knapweed buds are developing.

Mating takes place shortly after emergence and continues for 3-4 weeks. Females start laying eggs 3 days after mating. The eggs hatch in 3-4 days, and the larvae burrow into the ovary of the flower. The larvae cause the plant to form a gall that the larvae nearly consume, leaving only a very thin tissue. The second generation overwinters in the seedhead.

Identification:

Adults: Adult flies are 1-3 mm long. The flies have black bodies, with black wing bands striped in a distinctive "UV" pattern. Females have a prominent ovipositor [2-14].

Larvae and pupae: Larvae are milky to opaque white with a flat brown surface on the posterior end. The pupae are brown inside the seed hull. Pappus hair may still be attached, causing the gall at first glance to appear as a seed hull [2-15].



2-1 Agapeta zoegana adult root boring moth



2-3 Pterolonche inspersa adult root boring moth



2-6 Larinus minutus adult seedhead weevil



2-2 Agapeta zoegana larva and root feeding damage



2-4 Cyphocleonus achates adult root boring weevil



2-5 Cyphocleonus achates larvae and root feeding damage



2-7 Larinus minutus adult emergence hole in knapweed seedhead



2-8 Larinus obtusus adult seedhead weevil



2-9 Bangasternus fausti adult seedhead weevil



2-10 Metzneria paucipunctella adult seedhead moth



2-11 Metzneria paucipunctella larva and seedhead feeding damage



2-12 Urophora affinis adult seedhead fly on plant gall







2-13 *Urophora affinis* larva and gall in knapweed seedhead



2-14 Urophora quadrifasciata adult seedhead fly



2-15 Urophora quadrifasciata gall compared to knapweed seed



2-16 Chaetorellia acrolophi adult seedhead fly



2-18 Sphenoptera jugoslavica adult root boring beetle on diffuse knapweed



2-17 Terellia virens adult seedhead fly



2-19 Sphenoptera jugoslavica larva and root feeding damage





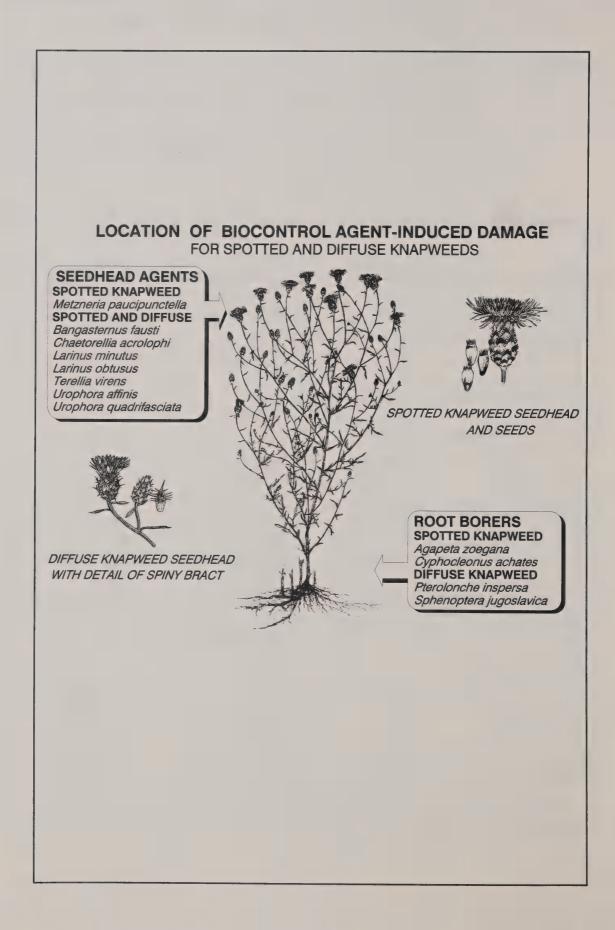


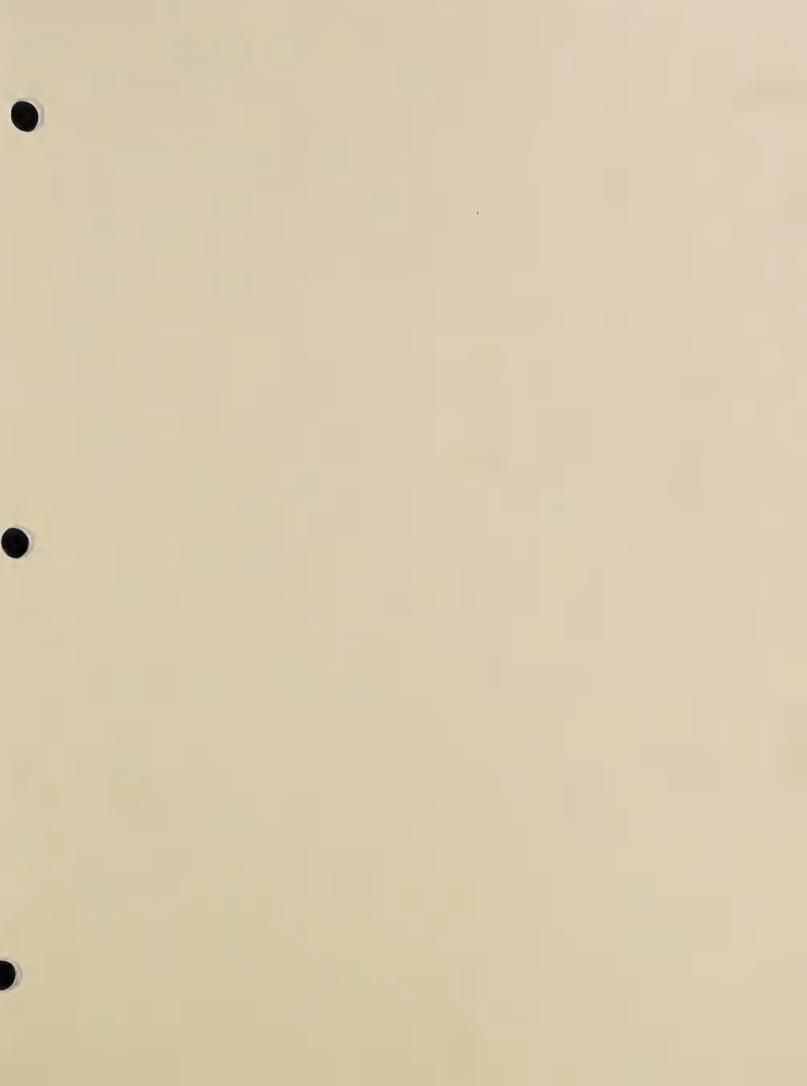
APPENDIX 3: SUMMARY OF DIFFUSE AND SPOTTED KNAPWEED BIOCONTROL AGENTS (1993-1994)

Introduction

Use this appendix as a quick reference to the biocontrol agents currently in use for control of diffuse and spotted knapweeds. The table includes a pronunciation guide for the scientific names, as well as a summary of each insect's classification. You will also find information on the range, release history, and life cycle of each biocontrol agent. APHIS has used other agents in the past and plans to use additional agents in the future, but you will be working with one or more of the insects listed in this appendix when you establish field insectary sites. To see where each biocontrol agent damages the knapweed plants, refer to page 6.2.

Species:	Pronunciation:	Order: family:	Native range:	1st yr rel. U.S.:	# Gen./ yr.:	Overwintering stage:
Agapeta zoegana (L.) (Root-boring moth)	Ag-a-pe'-ta zō-ee-gay'-na	Lepidoptera: Cochylidae	Europe	1984	1-2	Larva
Bangasternus fausti Reitter (Seedhead weevil)	Bang-a-stern'-iss fowst'-ee-eye	Coleoptera: Curculionidae	Southern Europe	1992	1	Adult
Chaetorellia acrolophi (White and Marquardt) (Seedhead fly)	Kee-toe-ree'-lee-a ack-row-loaf'-eye	Diptera: Tephritidae	Europe	1992	2?	Larva
Cyphocleonus achates Fabricius (Root-boring weevil)	Sy-foe-klee-own'-iss ah-kay'teez	Coleoptera: Curculionidae	Eurasia	1987	1	Larva
Larinus minutus (Gyllenhal) (Seedhead weevil)	La-rine'-iss my-noot'-iss	Coleoptera: Curculionidae	Eurasia	1991	1	Adult
Larinus obtusus (Gyllenhal) (Seedhead weevil)	La-rine'-iss ob-two'-sus	Coleoptera: Curculionidae	Eurasia	1993	1	Adult
Metzneria paucipunctella (Zeller) (Seedhead moth)	Mets-nair'-ee-a poss-ee-punk-tell'-a	Lepidoptera: Gelechiidae	Europe	1971	1	Larva
Pelochrista medullana Staudinger (Root-boring moth)	Pay'-low-kris-ta med'-u-lan-a	Lepidoptera: Tortricidae	Eurasia	1983	1	Larva
Pterolonche inspersa Staudinger (Root-boring moth)	Ter'-o-long-kee in-sper'-sa	Lepidoptera: Pterolonchidae	Eurasia	1986	1	Larva
Sphenoptera jugoslavica Obenberger (Root-boring beetle)	Sfen-op-ter-a yu'-go-slav'-i-ka	Coleoptera: Buprestidae	Europe	1979	1	Larva
Terellia virens (Loew) (Seedhead fly)	Ta-ree'-lee-a veer'-ins	Diptera: Tephritidae	Eurasia and N. Africa	1992	2?	Larva
Urophora affinis Frauenfeld (Seedhead gall fly)	Your-off'-urr-a a-fin'-iss	Diptera: Tephritidae	Eurasia	1971	1-2	Larva and pupa
Urophora quadrifasciata (Meigen) (Seedhead gall fly)	Your-off'-urr-a kwad-ri-faw-see-ay'-ta	Diptera: Tephritidae	Eurasia	1988	2	Larva and pupa







APPENDIX 4: OPERATION OF TRANSPAK II GLOBAL POSITIONING SYSTEM (GPS) UNITS

Introduction

To determine the locations of Field Insectary Sites (FIS), use the TRANSPAK II GPS Unit. Detailed instructions for the operation of Transpaks are provided by the manufacturer. Please review these instructions before operating the unit. All Transpak users must operate the units in the same manner to provide uniformity in the data. Be sure that the unit you are using is configured in the following way:

SETUP

LAND / AUTO
MG / ENGLISH /DMD
LOC = UTC +/- # / ASCII
NAD - 27 CONUS / OOS

Before visiting a site to be determined through global positioning, note the site's elevation from a topographical map. Three dimensional fixes are best, but it is sometimes necessary to enter the elevation manually if too few satellites are available to get a 3D fix. Error in latitude/ longitude readings will occur in 2D mode if the elevation has changed significantly since the last 3D fix. The accuracy of the 2D solution depends on the accuracy of the estimated elevation! Review section POS (pages 16-19) in the operation and maintenance guide for more information on manually entering elevations and Auto/2D/3D operation of Transpak II GPS units. Obtain maps from your records to assist you in locating the FIS in preparation for site visits. The local APHIS office should have copies of FIS maps and the coordinates (Township, Range, and Section #) for each FIS.

Saving GPS Determined Locations In Memory The Transpak II GPS unit is able to store 999 waypoints in memory. To save the current location, switch the mode indicator to WPT, toggle the L/R switch until the flashing cursor highlights FIX and flip the +/- switch. The current location will be saved as the next available waypoint. Record the waypoint number and site name in your field book or on the enclosed form and/or use the EDIT function to assign a label (site name) to the waypoint for identification. The Transpak II unit will not store elevation in memory. Read the elevation from the POS screen and record in your field notes along with waypoint number and site name. Review section WPT (pages 30-37) for more information regarding waypoint functions.

Reporting Waypoint Locations and Elevations Use the enclosed form for reporting waypoint latitude-longitudes and elevations. Include the township, range, and section coordinates for releases made before the 1992 field season. The township, range, and section coordinates are the current reference in the database for the site location and must be included in the report. New release locations (1992 and later) will not require township, range, and section data--only latitude-longitude derived by Transpak GPS.

Locating GPS Derived Biological Release Locations One of the features of the Transpak II GPS unit is its ability to navigate or steer the operator from the current position to a desired location. The desired location must be in the waypoint library for the unit to navigate to that point. To activate this feature, flip the mode switch to the NAV position. To navigate to a known point, key in the waypoint number at the TO blank on the NAV screen. The velocity, heading, range, and bearing are displayed on the NAV screen. Although this function is designed for

naval or aerial use, you can use it to locate positions on foot. It does take some practice, however, and you should attempt the procedure prior to actual field application. The following definitions are useful in the application of this function:

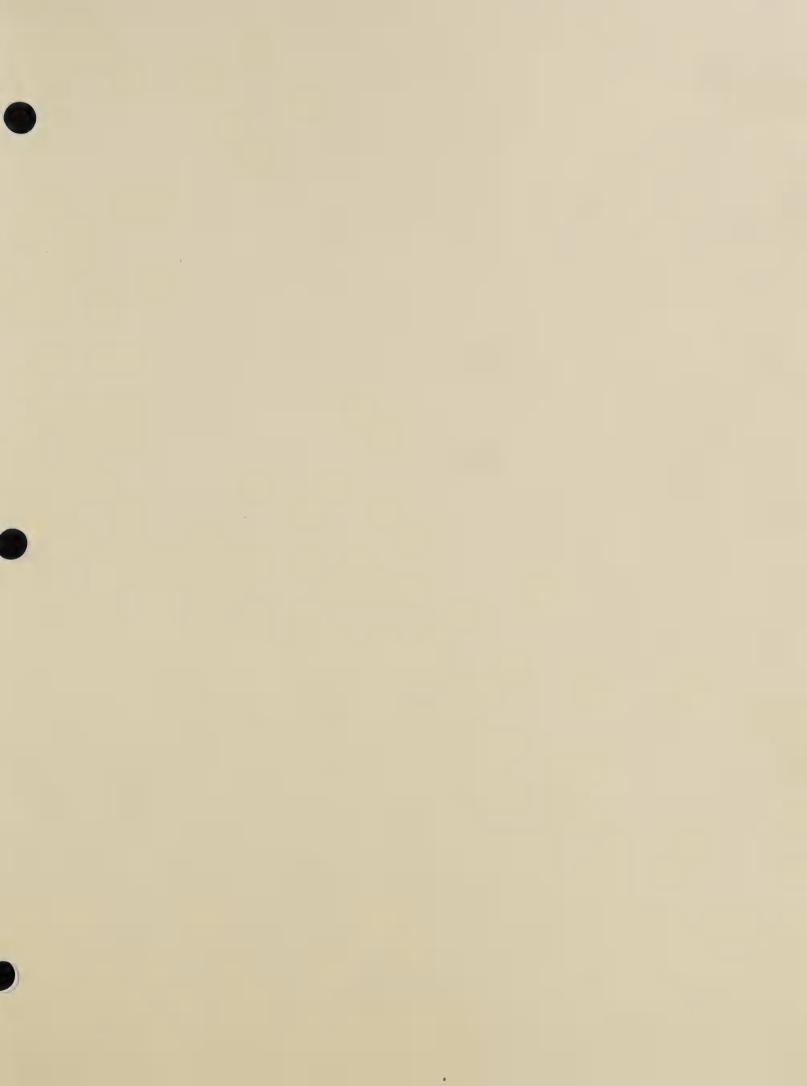
RANGE -- The distance from the present position to the desired location.

BEARING -- The direction to the desired location relative to magnetic north expressed as a compass reading in degrees.

HEADING -- The horizontal direction a moving ship, plane, or person is pointed, also expressed as a compass reading in degrees.

If the desired location is not in the waypoint library, you can key it in through the **EDIT** option of **WPT** mode (see pages 30-37 for more information). A hand-held compass may be useful in helping you locate biological release points. The **DIST** function is also useful in determining the distance and bearing from the present position to a release site location and is sometimes easier to use than the **NAV** function. Save the current position as a waypoint prior to using the **DIST** function. For more information on **NAV** and **DIST** functions, consult the Transpak II operation and maintenance guide (pages 20-25 and 28).

Please direct all questions or comments regarding the operation of Transpak II GPS units to the Bozeman Biological Control Facility.





APPENDIX 5: APHIS PROCESS TO RELEASE EXOTIC NATURAL ENEMIES OF WEEDS FOR ESTABLISHMENT AND REDISTRIBUTION

Introduction

This appendix summarizes the "Phase" concept of weed biological control. APHIS applies the "Phase" concept to the biological control of other weeds as well as spotted and diffuse knapweed. In Phase 1, initial releases of natural enemies are made into limited, protected, field insectaries. APHIS performs releases and management. Phase 2 involves cooperative establishment of field insectaries with materials from Phase 1 initial field insectaries. Technology is transferred from APHIS to cooperators, and management is by cooperators. In Phase 3, natural enemies are redistributed from Phase 2 field insectaries. Total management is by the cooperator.

Phase 1

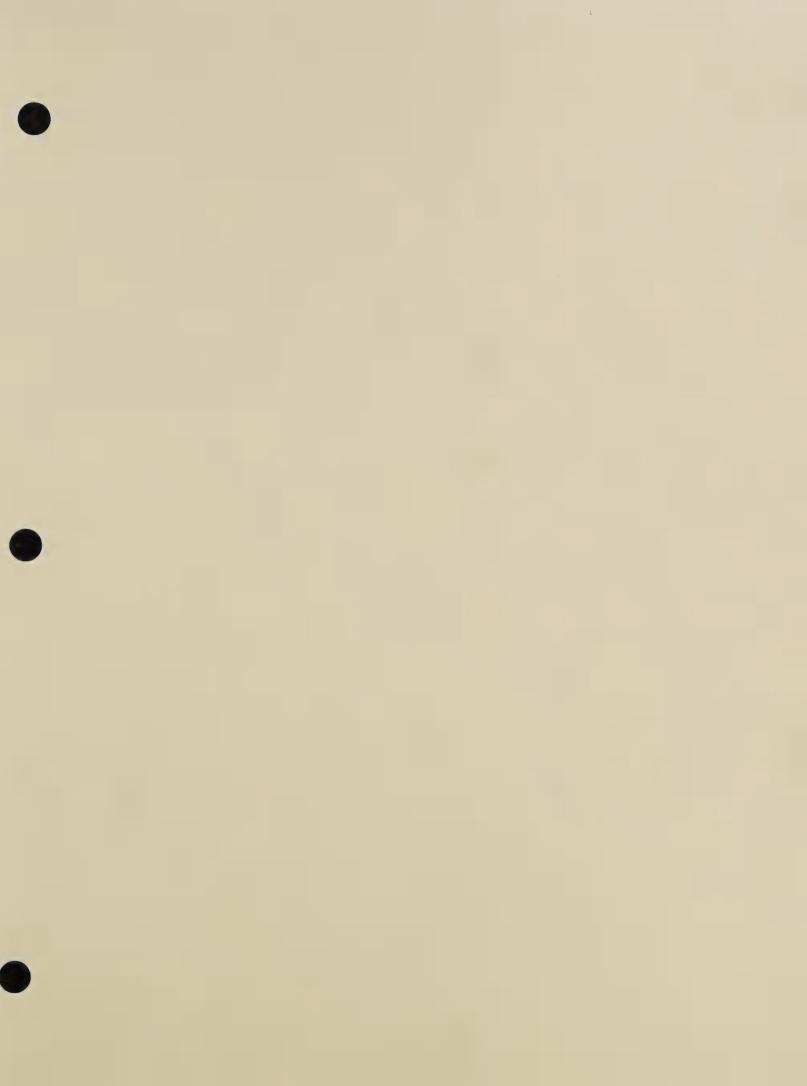
The introduction and release of exotic natural enemies for weed control involve initial releases of only a few organisms, at well defined and protected field sites, that would be free of any pesticide applications and open grazing. These initial sites would be carefully selected by APHIS with the assistance of various cooperators. These sites are limited in number depending on the availability of specimens collected from foreign sources and cleared through importation procedures. These sites may be in several States or only one State, possibly confined to only one county. APHIS' responsibility in Phase 1 is to protect and maintain these critical sites for several years in order to promote natural population increases of a particular species. The insects produced at such a location then become available for Phase 2. The time required between initial introduction and population development sufficient for redistribution to Phase 2 insectaries may be 3-5 years dependent upon species involved, initial numbers of agents released, and factors affection population development.

Phase 2

The establishment of field insectary sites (FIS) from initial Phase 1 releases would occur in each state that is infested by the target weed of concern. The establishment of these Phase 2 FIS is a joint cooperative effort by APHIS and state departments of agriculture and/or research cooperators. These sites will serve two purposes. Each site will be the source of additional natural enemies in 3 to 5 years for continued redistribution in Phase 3. These sites may also serve as demonstration plots, showing the potential impact of the natural enemy on the targeted weed in that particular State. During Phase 2, APHIS will serve as a source of information and techniques in support of State departments of agriculture and cooperators.

Phase 3

Natural enemies collected from Phase 2 FIS will be redistributed within each State. These releases may be directed at the county level for establishment of each species. Establishment of additional FIS within each county and/or at individual grower sites will be determined by the State department of agriculture and/or research cooperator. The collection and redistribution of natural enemies from the FIS developed in Phase 2 is the sole responsibility of the State departments of agriculture and/or research cooperators. At this time, commercial insectary operations and the general public could approach either responsible party for starter culture material and/or assisting in the redistribution efforts within that State.





APPENDIX 6: EXAMPLES OF FORMS

Introduction

This appendix provides you with examples of the following forms used for the DSK 6 Project:

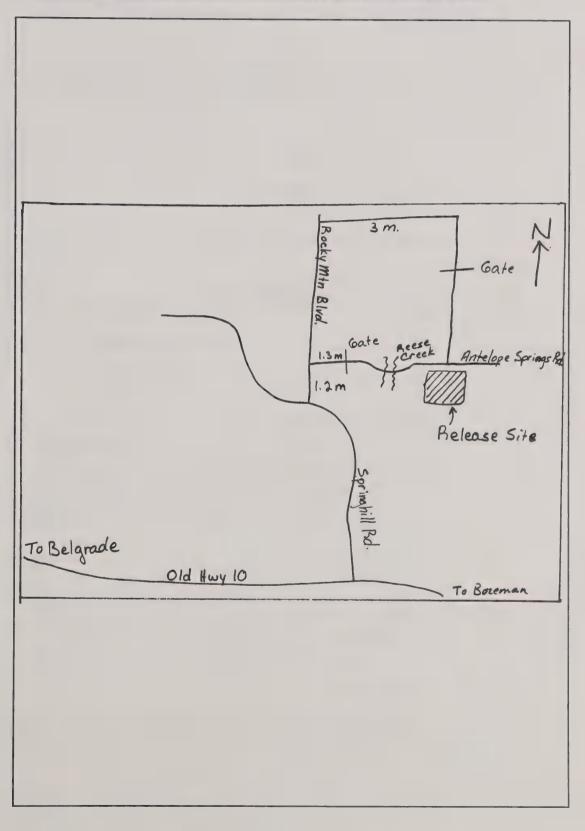
- USDA-APHIS BIOCONTROL OF WEEDS: FIELD INSECTARY SITE PRELIMINARY INFORMATION SHEET (FISPIS)
- BIOLOGICAL SHIPMENT RECORD--NON-QUARANTINE (Form AD-943)
- SUPPLEMENTAL DATA (Form AD-943A)
- Diffuse and Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT

Refer to the appropriate sections of the manual for specific instructions on completing these forms.

Examples Field Insectary Site Preliminary Information Sheet (FISPIS):

USDA-APHIS BIOCONTROL OF WEEDS: FIELD	INSECTARY SITE PRELIMINARY INFORMATION SHEET (FISPIS)
Target Weed: Leafy spurge X Diffuse/s	spotted knapweed (Release Code: 08035 - 07
Contact person: Name: John D. Greene	Legal landowner, if different (person or agency):
Name: John D. Greene Address: Gallatin Ca Courthouse	Name: Jane Wayne Address: 5099 Backy Mtn. Blvd.
City: Rozeman State: mT Zip: 99999	City: <u>Rozeman</u> State: <u>m7</u> Zip: <u>99999</u>
Phone: 406-555-555	Phone: 406-555-1111
SITE LOCATION:	
Site name: Antelope Sprinas	
atitude: #F #/ 32.4 N Longitude: W 02 3.7	:: T 2 S Range: R 6 E Sect.: 6 Otr-sect.: S W (derived from GPS?: X Yes No)
	heet, or attach a map, that includes road access to site 4
SITE CHARACTERISTICS (please check answ	er or supply requested information; if you are unable ion, please leave it blank.)
PHYSICAL	
1. Soil texture: Fine (clay) X Medium (cilt/loon	The Coarse (cand/grave)
2. Typical soil moisture regime: Well-drained	X Moderately well-drained Poorly drained
3. Hisk of spring flooding: X None Low-mod	derate (occasional years) High
5. Specific site topography: X Slight slope	Steep slope Level
(Is release site situated on a: hilltop or in	a valley?)
5. Is the slope lacing: N X S E 7%.	w (cneck two for combinations, e.g. SW)
3. Altitude (if known): 4950 ft (derived from GPS	S: _X_Yes No)
1. Soil texture: Fine (clay))-15" >20"
BIOLOGICAL	
Is wood infectation: Continuous V Intern	and the state of t
1. Size of weed infestation: 1 ac or less X 2	upted (weed patches separated by open or non-weed areas)
2. Weed density: X 0-40 stems/ft ² 40-100 st	ems/ft ² 100 or more stems/ft ²
 Vegetation cover (ground area shaded by plants):	0-25%
Typical weed height: <2 ft 2 ft or great	pted (weed patches separated by open or non-weed areas) 1-5 ac or more 100 or more stems/ft² 100 or more stems
i. Is weed flowering?: Yes No	V No. 1
(If present, are trees: Conifers X De	X No; in surrounding areas: X Yes No eciduous) Full Partial X None n the most abundant and conspicuous (dominant) plants:
. Shade from trees and shrubs in release site: F	ull Partial X None
What is the "natural" vegetation in the area, based or	n the most abundant and conspicuous (dominant) plants: Shrub Shrub-grassland K Grassland/prairie
rorest Savanna (frees and grassiand)	Shrub Shrub-grassland Grassland/prairie
CULTURAL	
Current land use: X Pasture Recreational	Roadside/right-of-way Idle cropland Other
(If Other, blease describe:	
. Herbicides applied within the last 2 (two) years?:(If Yes, list herbicide:	Month/year applied:
. Other treatments within last 12 (twelve) months?	Month/year applied:)
a. Mowing: Yes X No	c. Insecticides (e.g. grasshopper control): Yes 📈 No
(If Yes, what grazers: Cattle X Shee	c. Insecticides (e.g. grasshopper control): Yes K No d. Timber harvesting: Yes K No p or goats Horses)
OTHER.	<u> </u>
Accessible by vehicle within 1/4 mile (including 4WD Unauthorized access "controlled" (fences, etc.)?:) Yes No
. This release of biological control agents requires a 5	(live) year commitment, that includes:
a. No grazing from June through September, un	less release site is fenced
 b. No herbicide or insecticide applications d. Allowing periodic access by APHIS and coope 	c. Restricting access by unauthorized collectors
s the cooperator aware of, and willing to make this co	ommitment?:
s the deeperater arrange of, and willing to make this co	ions
X Yes No Yes, but with these restrict	

Field Insectary Site Preliminary Information Sheet (FISPIS) (back):



Form AD-943 (Biological Shipment Record - Non-Quarantine):

		34. FINAL DETERMINATION - Ger	OMB NO. 0518-0013 (EXP. 4/30/93) - sp., subsp., auth. 38. V.S.
U.S. Department of Agricultur BIOLOGICAL SHIPMENT RECORD — NOI		a.	M NC (see files)
SECTION	I – REPORT OF MA	TERIAL RELEASED OR SHIPPED	0000
1. FROM (Name & address of Shipper/Releaser)		CIAL - A. Gen., sp., subsp., auth.	M 3. SHIPPER / RELE ASER FILE NO. (see instructions)
USDA, APHIS, PPQ	Urop B. Order: F	shora spp.	BBCF - URAF 94- 04
Forestry Sciences Laboratory Montana State University Bozeman, MT 59717-0278		ra: Tephritidae	PArasite Weed feeder PRedator POllinator Microbial OTher
		Richard, USDA-APHIS	(Explain MI or OT):
Part A. From U.S. Field Collection (Collected for field to field recolonization or labors) 5. COLLECTION LOCALITY(S)-State, County, nearest To (If more than 2 collection sites, give State & County only) Montana, Coallatin Co., Gallatin Co.	story culture)	9. SOURCE FILE NOS. AD-942, AD-943: Nos. Part A Other: 10. COUNTRIES/REGION/STATE OF	aboratory Culture
6. DATES OF COLLECTION (m,d,y) 7. COLLECTORS (Names affiliations) 07/01/94 USDA-APHIS		11. ORIGINAL COLLECTORS (Names and affiliations)	12. NO. LAB GENERATIONS (At shipper/releaser location) Ft - Ft0 Ft1 + Ft0 Ft1 +
8. U.S. FIELD HOSTS/PREY AT COLLECTION A. Genus, species	IB. Stage/part attacked (see codes)	13. LABORATO RY HOST / PREY A. Genus, species	IB. Stage/part attacked (see codes)
Centaurea maculosa	SH		
14. SHIPPED TO (Name & address)	SECTION II - REI	PORT OF SHIPMENT 15. NO. & STAGES SHIPPED (use codes or	n reverse) 16. DATE SHIPPED (m,d,y)
John D. Greene		2000 A	07/01/94
Gallatin County Courthouse		17. SHIPPER'S REMARKS	18 SPECIMENS RETAINED
Bozeman, MT 99999		2 containers	BY SHIPPER
VIA: Federal Express		1000 A each	✓ No ☐ Yes nos.
07/00/04/ [5:0]	merged (Beneficials)	21. RECEIVER'S REMARKS	
17807		Received in good	
BY RECEIVER A. Dimmediate release (complete Seat VIII)	ab culture/study complete Blk. 24)	24. INTENDED LAB HOST / PREV -Gen.,	sp.
SECTION III – REPORT OF RELEASE/R	No release intended	See instructions on cover sheet: use Form	AD 9424 for more details
X Field Green	1	Field Greenhouse Gage	SITE 3
26. Locations (State, County, Montana, Ga	ulatin Co.	Other:	Field Greenhouse Cage Other:
feature, map coordinates) (Use AD-943A for more details: see instructions on cover sheet)			
27. Number & stages released (Use codes; see instructions for recording multiple releases.)	9	st]	[Est]
28 Dates of releases (m,d,y) (See instructions for recording multiple releases.) 7/02/9	4		
29. Target hosts/prey at release A. Primary - Genus, species Centaurea me	oculosa		
B. Other - Genus, species			
C. Families 30. Food (plant/animal/other) of target host/prey at release			
(1)	eene Galla	tin Co. Weed Supe	ervisor
Location marked with steel p			33. REPORTED BY A. Name John D. Greene B. Date (m,d,y) 07/02/94
Form AD-943 (5/90)			- DOCUMENTATION CENTER COPY

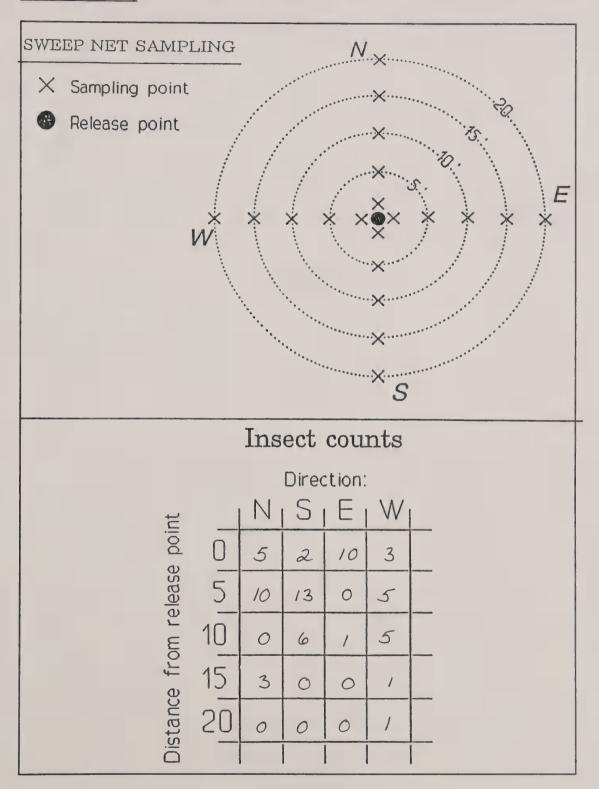
Form AD-943A (Supplemental Data):

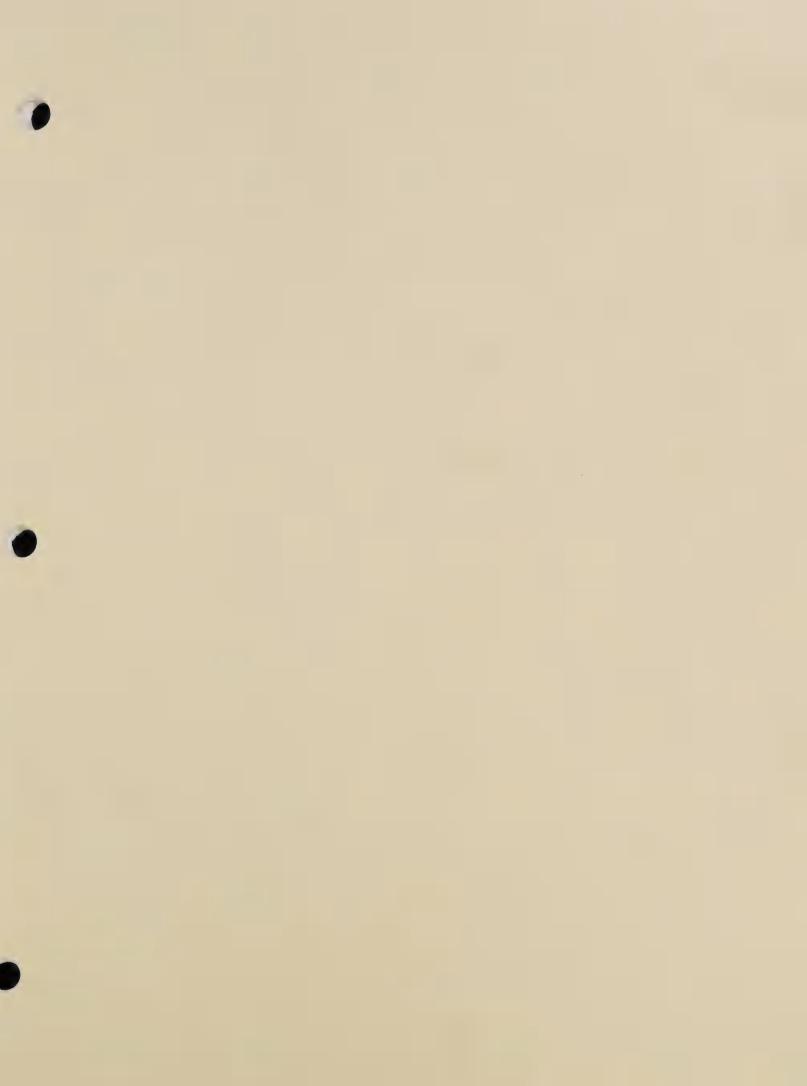
SUPPLEMENTAL	NOTE: • Do not	fold this sheet ove	r form when writing-c.	irbons will distort	entries.	Shipper's File Number (From AD 943)
DATA	• H addii	ional copies are n	eeded, photocopy and	staple to form.		BBCF-URAF94-04
Township, route no., Farmer's	Section	n A - RELEASE	SITE DETAILS, SIT			
Township, route no., ranners	1 To Belgrade	ase site.	0.1		TEME	* / F
	1	w —	-E C10	7	SKY	Cloudy
Frog's Hair Rd.	+	5	TIME OF R			,
			CONDITIO	N OF CROP FIEL	.D	
			CONDITIO	N OF RELEASE	MATERIAL	
Š			600	d		
ockrabbit Zone	2	nar	STAGE PRE	ANT TARGET H	OST/PREY	
266		OF M	TARGETH	OST/PREY ABU	NDANCE	
الم لا		2 7	ADDITION	AL HOST/PREV	PRESENT	
2		310.5m		AL 1103171 NE 1	, ar sen i	
THER COMMENTS						REPORTED BY & DATE
	Section B - DETAI	LS OF ADDITIO	NAL RELEASES (ALL	ach additional sh	eets as needed	
	Field Greenho	E 4		TE 5		SITE 6
Types of release Locations (State, County,	Other:		Other:		Dotne	
nearest Town or physical eature, map coordinates)						
Number and stages released See codes)	[Est]		[Est]		Est	
Dates of release (m,d,y)						
A. Primary - Genus, species						
3. Other - Genus, species						
. Families						
ood (plant/animal/other) of arget host/prey at release						
Released by						
Iternatives 1 and 2)			E RELEASES (Attac		ts as needed.)	SITE
Dates of release	Nos. Released (stages)	Dates of releas	Nos. Re	leased (*)	Dates of releas	e Nos. Reteased
A						
Counties			Locations		Dates of Releas	se No. Released (Stuges
MARKS						REPORTED BY
						A. Name

Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT:

Forestry S Bozema	DA-APHIS-PPQ n Biocontrol Facility Sciences Lab - MSU n, MT 59717-0278					
Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT						
Release Site Location: State: // cntana County Lea Site name: East Gate USGS coordinates: TION R R R Sect So No Qt Latitude: 4636 16 N Longitude: 1853 21.94 (GPS) derived: Y	tr-sect_5u)					
PIOCONTRO	A ACENTE BELLEAGED.					
Root boring moth Agapeta zoegana Seedhead moth Metzneria paucipunctella	Date of original release July 20, 1991					
Seedhead flies Urophora spp. Seedhead weevil Bangasternus fausti Seedhead weevil Larinus minutus Root boring weevil Cyphocleonus achates	Seedhead weevil Larinus obtusus Seedhead fly Chaetorellia acrolophi Seedhead fly Terellia virens Root boring moth Pelochrista medullana					
Root boring weevil Sphenoptera jugoslavica	Root boring moth Pterolonche inspersa					
f Sweeping: Visual observation of insect before sweeping?X X TOTAL number of agents sweeping: X X X X X X X X X						
Observer: S. D. Green & Affiliation: 45DH - HITTS - PTC,						
Observer: 5. 1). (1/10/10/10/10/10/10/10/10/10/10/10/10/10						
SAMPLIN SWEEPING: First, look over the release area to see if biocontrol innes in N, S, E, and W direction from release point (20 points to 5-in diameter sweep net, make four sweeps in front of you (bac hat the net moves vigorously through the vegetation as close to to insects present, then empty the net to release counted insects. Moveen sampled, then repeat over the remaining cardinal directions.	IG INSTRUCTIONS insects are visually apparent. Next, sweep five sampling points along fountal). For each line, begin as close to the release point as possible. Using ck and forth twice). Each net sweep should proceed in a downward arc, so the ground as possible. Carefully examine the net and count the biocontropute of the sampling procedure and a chart on which to record insect adaptation in a glass vial with 70% alcohol. Submit Agapeta adults as dressed as the sampling procedure and a chart on which to record insect accordance in a glass vial with 70% alcohol. Submit Agapeta adults as dressed as the sampling procedure and a chart on which to record insect accordance in a glass vial with 70% alcohol. Submit Agapeta adults as dressed as the sampling procedure and a chart on which to record insect accordance in the sampling procedure and a chart on which to record insect accordance in the sampling procedure and a chart on which to record insect accordance in the sampling procedure and a chart on which to record insect accordance in the sampling procedure and a chart on which the sampling procedure are sampling procedure and a chart on which the sampling procedure are sampling procedure.					
SAMPLIN SWEEPING: First, look over the release area to see if biocontrol ines in N, S, E, and W direction from release point (20 points to 5-in diameter sweep net, make four sweeps in front of you (bac hat the net moves vigorously through the vegetation as close to to insects present, then empty the net to release counted insects. Moveen sampled, then repeat over the remaining cardinal directions. Place adults of Larinus, Bangasternus, Sphenoptera and Cypho	insects are visually apparent. Next, sweep five sampling points along fountal). For each line, begin as close to the release point as possible. Using the and forth twice). Each net sweep should proceed in a downward arc, so the ground as possible. Carefully examine the net and count the biocontropuse 5 to 6 ft out and repeat the above steps. Continue until five points have A diagram of the sampling procedure and a chart on which to record insections of the sampling procedure and a chart on which to record insections in a glass vial with 70% alcohol. Submit Agapeta adults as drecimens to the Bozeman Biocontrol Facility along with this form. Send					
SAMPLIN SWEEPING: First, look over the release area to see if biocontrol ines in N, S, E, and W direction from release point (20 points to 5-in diameter sweep net, make four sweeps in front of you (bac hat the net moves vigorously through the vegetation as close to the insects present, then empty the net to release counted insects. Moveen sampled, then repeat over the remaining cardinal directions. Founts is provided on the back of this form. Place adults of Larinus, Bangasternus, Sphenoptera and Cypho pecimens. Label all vials with site name and date and send spenaximum of 5 adults of these agents. If you collect no specimen VISUAL: Sit quietly for a few minutes in the knapweed patch near	insects are visually apparent. Next, sweep five sampling points along for tal). For each line, begin as close to the release point as possible. Using ck and forth twice). Each net sweep should proceed in a downward arc, st the ground as possible. Carefully examine the net and count the biocombove 5 to 6 ft out and repeat the above steps. Continue until five points have A diagram of the sampling procedure and a chart on which to record inservedeous in a glass vial with 70% alcohol. Submit Agapeta adults as drecimens to the Bozeman Biocontrol Facility along with this form. Send as, return only the completed form. The release point and look for the insects. If you see none, then carefull as terms. Agapeta will often rest vertically with the stem and may look like					
SAMPLIN SWEEPING: First, look over the release area to see if biocontrol ines in N, S, E, and W direction from release point (20 points to 5-in diameter sweep net, make four sweeps in front of you (bac hat the net moves vigorously through the vegetation as close to the insects present, then empty the net to release counted insects. Moveen sampled, then repeat over the remaining cardinal directions. Founts is provided on the back of this form. Place adults of Larinus, Bangasternus, Sphenoptera and Cypho pecimens. Label all vials with site name and date and send spenaximum of 5 adults of these agents. If you collect no specimen of Sulla Sit quietly for a few minutes in the knapweed patch near and slowly move the plants aside to look under the leaves and on the dead leaf. Larinus and Bangasternus adults will be found on the SEEDHEAD COLLECTIONS: Collect seedheads for Urophora specimens.	insects are visually apparent. Next, sweep five sampling points along for tal). For each line, begin as close to the release point as possible. Using ck and forth twice). Each net sweep should proceed in a downward arc, sthe ground as possible. Carefully examine the net and count the biocombove 5 to 6 ft out and repeat the above steps. Continue until five points have A diagram of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice or the Bozeman Biocontrol Facility along with this form. Send us, return only the completed form. The release point and look for the insects. If you see none, then carefull the stems. Agapeta will often rest vertically with the stem and may look like the flower itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves.					
SAMPLIN SWEEPING: First, look over the release area to see if biocontrol ines in N, S, E, and W direction from release point (20 points to 5-in diameter sweep net, make four sweeps in front of you (bac hat the net moves vigorously through the vegetation as close to the insects present, then empty the net to release counted insects. Moveen sampled, then repeat over the remaining cardinal directions. For insects present, then empty the net to release counted insects. Moveen sampled, then repeat over the remaining cardinal directions. For insects present, then empty the net to release counted insects. Moveen sampled, then repeat over the remaining cardinal directions. For insecting the provided on the back of this form. Place adults of Larinus, Bangasternus, Sphenoptera and Cypho pecimens. Label all vials with site name and date and send spenaximum of 5 adults of these agents. If you collect no specimen of Security is the said to look under the leaves and on the dead leaf. Larinus and Bangasternus adults will be found on the SEEDHEAD COLLECTIONS: Collect seedheads for Urophora sport fit the plant. Randomly collect 200 seedheads (2 per plant) along	insects are visually apparent. Next, sweep five sampling points along for tal). For each line, begin as close to the release point as possible. Using ck and forth twice). Each net sweep should proceed in a downward arc, sthe ground as possible. Carefully examine the net and count the biocombove 5 to 6 ft out and repeat the above steps. Continue until five points have A diagram of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice of the sampling procedure and a chart on which to record inservice or the Bozeman Biocontrol Facility along with this form. Send us, return only the completed form. The release point and look for the insects. If you see none, then carefull the stems. Agapeta will often rest vertically with the stem and may look like the flower itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves. The procedure itself as well as stems and leaves.					

<u>Diffuse & Spotted Knapweed Biocontrol Agent RECOVERY AND SAMPLING REPORT (back):</u>







APPENDIX 7: HOST PLANT SPECIFICITY TESTING

Introduction

APHIS incorporates rigorous "safety testing" procedures into the process of classical biological control. You should feel free to assure any landowners or cooperators with whom you interact that the diffuse and spotted knapweed biocontrol agents described in this manual will not attack any economically important plants or native plants outside of the genus *Centaurea*.

TAG Evaluation

Any foreign biocontrol agent being considered for importation and release against diffuse and spotted knapweed first must be evaluated by a multi-agency Technical Advisory Group (TAG). TAG considers whether the organism in question is a potential threat to U.S. crop, ornamental, and native plants, and then recommends whether or not the agent should be introduced. USDA-APHIS, acting on a positive decision by TAG, then issues permits for the importation and release of the biocontrol agent.

Most of the evidence considered by TAG consists of "screening" or host-specificity experiments with the biocontrol agent in question. The International Institute of Biological Control (IIBC) in Switzerland or the USDA-ARS laboratory in Montpelier, France, tests for host-specificity of biocontrol insects. For some insects, USDA-ARS or university laboratories in this country may conduct additional tests under quarantine conditions. Entomologists test a variety of plant species, including diffuse and spotted knapweed and related species, plants reportedly eaten by insects related to the species being examined, and a range of crop and ornamental plants. The entomologists try to include a number of native species related to diffuse and spotted knapweed. Tests assess the "ability" of plant species to support: (1) adult insect feeding, if relevant; (2) egg deposition by the adult female insect; and (3) survival and development of the larval insect (i.e., completion of the insect's life cycle). The last factor is probably the most important in determining the likelihood of a biocontrol agent feeding on a plant species under field conditions.

Taxonomy of Centaurea

Botanists have not fully resolved the taxonomy of the genus *Centaurea*. *C. maculosa*, spotted knapweed, belongs to the difficult species group of *C. paniculata* L. consisting of 30 differentiated species. *C. diffusa* (diffuse knapweed), although in a different species group, is closely related and belongs to the same subgenus *Acrolophus*. It is debated whether or not that the genus *Centaurea*, which consists of around 500 species, developed in the Palearctic and only entered North America recently. *C. maculosa* in North America may have originated in Southern Russia and entered North America as seed in recent times.

Botanists are not in agreement if *C. americana* and *C. rothrockii* are native to North America. Some botanists feel that these two species are so closely related that they should be considered to be varieties of the same species, *Plectocephalus americanus*. The genus *Plectocephalus* consists of nine species found largely in South America. One theory proposes that these two species of knapweed were introduced from South America.

Agapeta zoegana Agapeta zoegana, a root-feeding moth, received approval in 1984 for U.S. release. European host-specificity tests show that this species feeds only on a few closely related Centaurea species: C. maculosa, C. arenaria, C. diffusa, C. vallesiaca, and C. nigrescens.

Bangasternus fausti Bangasternus fausti, a seedhead weevil, received approval in 1991 for U.S. release. European host-specificity tests show that this species' development is restricted to Centaurea diffusa and C. maculosa.

Chaetorellia acrolophi Chaetorellia acrolophi, a seedhead fly, received approval in 1990 for U.S. release. European host-specificity tests show that this species is mainly restricted to the subgenus Acrolophus in the genus Centaurea. Other plants that supported limited larval development were C. jacea, C. nigra, C. solstitialis, and C. calcitrapa.

Cyphocleonus achates

Cyphocleonus achates, a root boring weevil, received approval in 1987 for U.S. release. European field surveys found C. achates only associated with Centaurea diffusa and C. maculosa. European host-specificity tests show that C. achates is closely associated with Centaurea, subgenus Acrolophus.

Larinus minutus Larinus minutus Gyll., a seedhead weevil, received approval in 1991 for U.S. release. European host specificity tests show that L. minutus is restricted to the subgenera Acrolophus and Calcitrapa of the genus Centaurea. This weevil was reported to attack and develop on C. arenaria, C. calcitrapa, C. diffusa, C. iberica and C. maculosa.

Larinus obtusus

Larinus obtusus, a seedhead weevil, received approval in 1993 for U.S. release. European host specificity tests show that L. obtusus belongs to a group of restricted Larinus species that feed only on a few host plants. European collections of this weevil came only from Centaurea maculosa, C. jacea, and C. phrygia, all belonging to the subgenera Acrolophus or Jacea. The larval development tests clearly indicate this weevil is restricted to these two subgenera of the genus Centaurea.

Metzneria paucipunctella Metzneria paucipunctella, a seedhead moth, received approval in 1971 for U.S. release. European collection of M. paucipunctella was exclusively restricted to Centaurea stoebe L. (now called C. maculosa Lam.). Oviposition tests found that M. paucipunctella will lay eggs on C. diffusa and C. paniculata. Larval development was not recorded from these plants.

Pelochrista medullana Pelochrista medullana, a root boring moth, received approval in 1983 for U.S. release. European testing found that P. medullana development is restricted to Centaurea diffusa and C. maculosa.

Pterolonche inspersa

Pterolonche inspersa, a root boring moth, received approval in 1986 for U.S. release. European host specificity testing found that P. inspersa prefers Centaurea diffusa, but can feed on other species in the same genus. The plants fed upon were Centaurea maculosa, C. rhenana Boreau, C. vallesiaca, C. cineraria, C. friderici, C. paniculata, and C. diffusa. Centaurea americana and C. rothrockii were not tested because they are summer annuals and P. inspersa overwinters in live roots to complete development.

Sphenoptera jugoslavica

Sphenoptera jugoslavica, a root boring beetle, received approval in 1979 for U.S. release. European field surveys for host specificity found S. jugoslavica almost exclusively attacking Centaurea diffusa. A population of S. jugoslavica was found in a group of plants tentatively identified as a hybrid between C. diffusa and C. jurineaefolia Boris. Host specificity is limited in that the larvae cannot tolerate plant growth during the egg and first instar period of S. jugoslavica. The growing rosette crushes the egg or larvae. C. diffusa matches the criterion of arrested rosette growth during that oviposition time.

Terellia virens

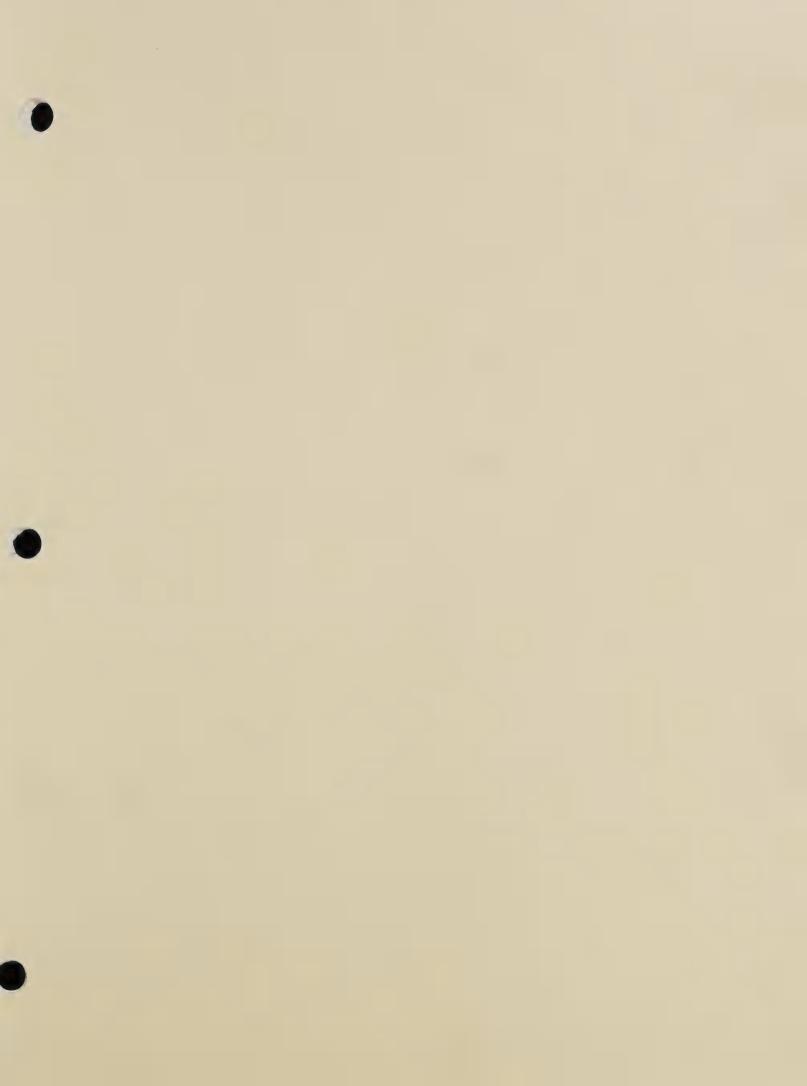
Terellia virens, a seedhead fly, received approval in 1992 for U.S. release. European host specificity tests demonstrated T. virens did not attack any plants outside the subgenus Acrolophus in the genus Centaurea except C. nigrescens. The North American biotype of C. maculosa reached the highest attack rate next to the likewise tetraploid C. micianthos. The T. virens of Austrian and Hungarian origin is highly specialized on Centaurea species and shows a clear preference for plants in the subgenus Acrolophus.

Urophora affinis

Urophora affinis, a seedhead fly, received approval in 1971 for U.S. release. European host specificity tests found that *U. affinis* is restricted to oviposition and development in three *Centaurea* species in the subgenus *Acrolophus*. The *Centaurea* species were *C. diffusa*, *C. maculosa*, and *C. paniculata*. The hybrid *C. jacea-maculosa* was accepted for oviposition.

Urophora quadrifasciata

Urophora quadrifasciata, a seedhead fly, received approval in 1988 for U.S. release. Host specificity tests conducted in the U.S. found that *U. quadrifasciata* does not attack safflower, *Carthamus tinctorius* and two native knapweeds *Centaurea rothrockii* and *C. americana*.



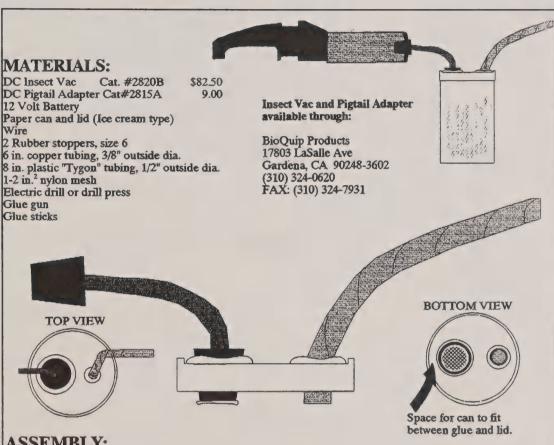


APPENDIX 8: COLLECTION VACUUM

Introduction

This appendix provides you with instructions on how to assemble a modified insect vacuum for collecting Agapeta zoegana.

Collection Vacuum for Agapeta zoegana:



ASSEMBLY:

- Cut two holes in paper can lid, one a bit larger than the small end of a #6 rubber stopper, and one a bit smaller than the
- plastic tubing.

 Wrap the plastic tubing with wire to support it, taking several wraps about 1" from one end. Bend the tubing in a shallow arc, taking care not to crimp it.
- Bend the copper tubing in an arc, so that the ends are at 90° angles to each other, and try not to crimp too deeply in any one spot. Drill 3/8" holes in each of the rubber stoppers, and work a stopper onto each end of the copper tubing (it should be a tight fit).
- Carefully work the plastic tubing and one stopper into their respective holes in the lid. Using the hot glue gun, place a large bead of glue around the plastic tubing and the stopper on the top of the lid. After the glue cools, place another bead around the plastic tubing and the stopper on the bottom of the lid. This will secure the tubes and ensure an airtight seal.
- Cut the mesh material the size of the small end of the stopper, and hot glue it to the bottom of the stopper in the lid. Or, if your copper tubing extends below the bottom of the stopper, cut a larger piece of mesh and use a rubber band to secure it to the tubing. Either method will prevent the vacuum from pulling insects into its motor, but if you use a rubber band to secure the mesh, check it periodically, as they tend to work off.
- Place a paper towel inside the can before you begin collecting to give the moths a soft place to land and to hang during transport.





INDEX

A - C

```
Acrolophus, 10.1-10.3
Agapeta zoegana,
     classification of, 6.1
     host specificity of, 10.2
     identification of, 5.1
     life cycle of, 5.1
     native range of, 6.1
     overwintering stage of, 6.1
     photograph of, 5.1, [2-1], [2-2]
     pronunciation of, 6.1
Alcohol,
     ethyl, 2.8-2.13, 2.19-2.25, 3.21-3.26
     isopropyl, 2.8-2.13, 2.19-2.25, 3.21-3.26
Bags, plastic,
     garbage, 3.13
     resealable, 2.23
Bangasternus fausti,
     classification of, 6.1
     host specificity of, 10.2
     identification of, 5.2
     life cycle of, 5.2
     native range of, 6.1
     overwintering stage of, 6.1
     photograph of, 5.2, [2-9]
     pronunciation of, 6.1
Battery, heavy duty 12-volt, 3.13
Beads, styrofoam, 2.7-2.13, 3.6, 3.9, 3.11, 3.14, 3.16, 3.19-3.25
Bed sheets, 2.17-2.18, 3.5-3.6
Bearing, definition of (relating to operation of Transpak II GPS units), 7.2
Black light, 2.17-2.18, 3.5-3.6
Blue ice, 2.7-2.13, 3.5-3.11, 3.14-3.17, 3.19-3.25
Bouquets, 3.12
Box, cardboard, 3.5, 3.7, 3.9, 3.11, 3.15, 3.17
Bozeman Biological Control Facility (BBCF), 1.3, 1.6, 2.1, 2.6
Buprestidae, 6.1
Cage, 10'x10', 3.13
Calcitrapa, 10.2
Carthamus tinctorius, 10.3
Cartons, shipping, 3.5-3.6
```

```
Centaurea,
      americana, 10.1, 10.3
     arenaria, 10.2
     calcitrapa, 10.2
     cineraria, 10.3
     diffusa, 1.1, 4.1, [1-6], [1-7], 10.1-10.3
     friderici, 10.3
     iberica, 10.2
     jacea, 10.2
     jacea-maculosa, 10.3
     jurineaefolia, 10.3
     maculosa, 1.2, 4.1, [1-1], [1-2], 10.1-10.3
     micianthos, 10.3
     nigra, 10.2
     nigrescens, 10.2-10.3
     paniculata, 10.1-10.3
     phrygia, 10.2
     rhenana, 10.3
     rothrockii, 10.1, 10.3
     solstitialis, 10.2
     stoebe, 10.2
     vallesiaca, 10.2-10.3
Chaetorellia acrolophi,
     classification of, 6.1
     host specificity of, 10.2
     identification of, 5.3
     life cycle of, 5.2
     native range of, 6.1
     overwintering stage of, 6.1
     photograph of, 5.3, [2-16]
     pronunciation of, 6.1
Clippers, 3.7, 3.10
Cochylidae, 6.1
Code, release, 2.5
Coleoptera, 6.1
Commitment, 5-year, 2.3, 3.4, 9.2
Container,
    gallon, 3.7-3.9
    quart, 3.6, 3.11, 3.16
Cooler, 2.7-2.13, 3.5-3.11, 3.14-3.17, 3.19-3.25
Curculionidae, 6.1
```

```
Cyphocleonus achates.
     classification of, 6.1
     host specificity of, 10.2
     identification of, 5.3
     life cycle of, 5.3
     native range of, 6.1
     overwintering stage of, 6.1
     photograph of, 5.3, [2-4]
     pronunciation of, 6.1
D - F
Damage, biocontrol agent-induced, 6.2
Density, stand, 2.3
Diffuse and Spotted Knapweed Biocontrol Agent Recovery and Sampling Report,
     example of, 9.6
     filling out, 2.27
Diffuse knapweed,
     description of, 4.1
     photographs of, [1-6], [1-7]
     taxonomy of, 10.1
Diptera, 6.1
DIST function, 7.2
DSK Project,
     economics, 1.2
     goal and objectives, 1.3
     history of, 1.1
     length of, 1.3
     roles and responsibilities for, 1.5
EDIT function, 7.1-7.2
Ethyl alcohol, 2.8-2.13, 2.19-2.25, 3.21-3.26
Exposure, 2.3, 3.3
Field Insectary Site (FIS),
    establishing a Phase 1 FIS.
         collecting samples of biocontrol agents, 2.17
         filling out a FISPIS, 2.5
         filling out a Diffuse and Spotted Knapweed Biocontrol Agent Recovery and Sampling Report,
         2.27
         filling out forms AD-943 and AD-943A, 2.15
         overview of, 2.1
         purpose of, 2.1
         releasing biocontrol agents, 2.7
         selecting a suitable site, 2.3
         when to establish, 2.1
```

```
Field Insectary Site (FIS) (continued),
      establishing a Phase 2 FIS,
          collecting biocontrol agents from a Phase 1 FIS for redistribution to a Phase 2 FIS, 3.5
          overview of, 3.1
          purpose of, 3.1
          releasing biocontrol agents, 3.19
          selecting a suitable site, 3.3
          when to establish, 3.1
Field Insectary Site Preliminary Information Sheet (FISPIS),
     example of, 9.2, 9.3
     filling out, 2.5
     functions of, 2.5
Field size, 2.3, 3.3
Form AD-943,
     example of, 9.4
     filling out, 2.15
Form AD-943A,
     example of, 9.5
     filling out, 2.16
Forms, examples of, 9.1-9.7
G-L
Galls, 3.12
Garbage bags, 3.13
Gelechiidae, 6.1
Gloves, heavy-duty, 3.12-3.13
Grazing, 2.3, 3.3
Hammer, sledge, 2.17, 3.5
Hansen, Richard, 1.3, 1.6
Heading, definition of, (relating to operation of Transpak II GPS units), 7.2
Herbicides, 2.3, 3.3
Insecticides, 2.3, 3.3
Insect.
     pin, 2.7, 2.18, 3.20, 3.24-3.25
    vacuum,
         modified, 2.17, 3.5, 11.1
         portable, 3.13-3.14
Isopropyl alcohol, 2.8-2.13, 2.19-2.25, 3.21-3.26
Jacea, 10.2
```

```
Knapweed,
     diffuse, 4.1, [1-6], [1-7]
     spotted, 4.1, [1-1], [1-2]
Land ownership, 2.3, 3.3
Lang, Ronald, 1.3, 1.6
Larinus,
     minutus,
          classification of, 6.1
          host specificity of, 10.2
          identification of, 5.4
          life cycle of, 5.4
          native range of, 6.1
          overwintering stage of, 6.1
          photograph of, 5.4, [2-6], [2-7]
          pronunciation of, 6.1
     obtusus.
          classification of, 6.1
          host specificity of, 10.2
          identification of, 5.5
          life cycle of, 5.4
          native range of, 6.1
          overwintering stage of, 6.1
          photograph of, 5.5, [2-8]
          pronunciation of, 6.1
Lepidoptera, 6.1
Life tweezers, 3.8-3.9, 3.16
Location of biocontrol agent-induced damage, 6.2
M - P
Masking tape, 3.5-3.7, 3.10-3.11, 3.14-3.16
Metzneria paucipunctella,
     classification of, 6.1
    host specificity of, 10.2
    identification of, 5.5
    life cycle of, 5.5
    native range of, 6.1
```

overwintering stage of, 6.1

pronunciation of, 6.1

Montana State University, 1.6

photograph of, 5.5, [2-10], [2-11]

Modified insect vacuum, 2.17, 3.5, 11.1

Mission Biological Control Laboratory (MBCL), 1.3, 1.5

```
NAV function, 7.1-7.2
 Net, sweep, 2.19-2.22, 2.24-2.25
 Officers-in-charge (OIC's), 1.3, 1.5, 2.6
 Ownership, land, 2.3, 3.3
 Pan, flat, 3.9, 3.16
 Paper towels, 3.5-3.6
 Parker, Paul, 1.3, 1.5
 Pelochrista medullana,
      classification of, 6.1
      host specificity of, 10.2
      identification of, 5.6
      life cycle of, 5.5
      native range of, 6.1
      overwintering stage of, 6.1
      pronunciation of, 6.1
 Pencils, 2.9-2.11, 2.13, 2.17, 2.19-2.25, 3.5, 3.7, 3.10, 3.15
 Pesticide use, 2.3, 3.3
Phase,
     concept of weed biological control,
     1, 8.1
     2, 8.1
     3, 8.1
Pin, insect, 2.7, 2.18, 3.20, 3.24-3.25
Plectocephalus americanus, 10.1
Portable insect vacuum, 3.13-3.14
Project Leader, 1.3, 1.5
Pterolonche inspersa,
     classification of, 6.1
     host specificity of, 10.3
     identification of, 5.6
     life cycle of, 5.6
     native range of, 6.1
     overwintering stage of, 6.1
     photograph of, 5.6, [2-3]
     pronunciation of, 6.1
Pterolonchidae, 6.1
R-T
Racks, screen, 3.13
Range, 2.5, 2.15, 9.2
    definition of, (relating to operation of Transpak II GPS units), 7.2
Release code, 2.5
```

```
Richard, Robert, 1.3, 1.6
Roles and Responsibilities, 1.5-1.6
Rubber bands, wide, 3.12
Samples, voucher, 2.7-2.13, 3.19-3.25
Screen racks, 3.13
Screens, graduated, 3.8
Section, 2.5, 2.15, 9.2
Seedheads, 2.21, 2.23, 3.7, 3.10, 3.12-3.13, 3.15
Sheets, bed, 2.17-2.18, 3.5-3.6
Shoot tips, 3.7, 3.10
Sledge hammer, 2.17, 3.5
Soil type, 2.3, 3.3
Sphenoptera jugoslavica,
     classification of, 6.1
     host specificity of, 10.3
     identification of, 5.7
     life cycle of, 5.6
     native range of, 6.1
     overwintering stage of, 6.1
     photograph of, 5.7, [2-18]
     pronunciation of, 6.1
Spotted knapweed,
     description of, 4.1
     photographs of, 4.1, [1-1], [1-2]
     taxonomy of, 10.1
Stake, metal, 2.7-2.13, 2.17, 3.5, 3.19-3.25
Stand density, 2.3, 3.3
State plant health directors (SPHD's), 1.3, 1.5, 2.6
State project coordinators, 1.3, 1.5
Styrofoam beads, 2.7-2.13, 3.6, 3.9, 3.11, 3.14, 3.16, 3.19-3.25
Sunlight, 2.3, 3.3
Sweep net, 2.19-2.22, 2.24-2.25
Tape, masking, 3.5-3.7, 3.10-3.11, 3.14-3.16
Tarp, 3.12
Technical Advisory Group, 10.1
Tephritidae, 6.1
Terellia virens,
    classification of, 6.1
    host specificity of, 10.3
    identification of, 5.8
    life cycle of, 5.8
    native range of, 6.1
    overwintering stage of, 6.1
    photograph of, 5.8, [2-17]
    pronunciation of, 6.1
```

```
Testing, host plant specificity, 10.1-10.3
Tortricidae, 6.1
Towels, paper, 3.5-3.6
Township, 2.5, 2.15, 9.2
Transpak II GPS unit,
     operation of, 7.1-7.2
Trash, 3.8-3.9
Tweezers, life, 3.8-3.9, 3.16
U-W
Ultraviolet (UV) light, 2.17-2.18
Urophora,
     affinis,
          classification of, 6.1
          host specificity of, 10.3
          identification of, 5.9
          life cycle of, 5.8
         native range of, 6.1
         overwintering stage of, 6.1
         photograph of, 5.8, [2-12]
         pronunciation of, 6.1
     quadrifasciata,
         classification of, 6.1
         host specificity of, 10.3
         identification of, 5.9
         life cycle of, 5.9
         native range of, 6.1
         overwintering stage of, 6.1
         photograph of, 5.8, [2-12]
         pronunciation of, 6.1
UV (ultraviolet) light, 2.17-2.18, 3.5-3.6
Vacuum,
     modified insect, 2.17, 3.5, 11.1
    portable insect, 3.13-3.14
Vial, glass screw cap, 2.8-2.11, 2.13, 2.19-2.22, 2.24-2.25
Voucher samples, 2.7-2.13, 3.19-3.25
Waypoint, 7.1-7.2
WPT mode, 7.1-7.2
```

BIOLOGICAL CONTROL OF DIFFUSE AND SPOTTED KNAPWEED Comment Sheet

Directions: Use this sheet to suggest an improvement or to identify a problem in the content of the manual. To mail, please follow the directions on the next page.

BCO 05/95-01

AFTER COMPLETION, FOLD ON THE DOTTED LINES WITH THE ADDRESS SIDE OUTWARD. STAPLE OR TAPE TO CLOSE, AFFIX POSTAGE, AND DROP IN THE MAIL. Professional Development Center USDA-APHIS-R&D 7340 Executive Way, Suite A Frederick, MD 21701

Attn: Bruce Attavian